FY 1992 ANNUAL REPORT

BIOMEDICAL ENGINEERING AND INSTRUMENTATION PROGRAM

NATIONAL CENTER FOR RESEARCH RESOURCES

NATIONAL INSTITUTES OF HEALTH

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ANNUAL REPORT FOR FY 1992

Dr. Murray Eden, Director

BEIP RESEARCH PROJECTS (Z01 RR)

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10001-24 BEI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Pharmacokinetics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert L. Dedrick, Ph.D.	Chief, CHES	BEIP, NCRR
Cynthia Sung, Ph.D.	Staff Fellow	BEIP, NCRR
Paul F. Morrison, Ph.D.	Physical Scientist	BEIP, NCRR

COOPERATING UNITS *(if any)*

NM, CC; DR, CC; NTP, NIEHS; SNB, NINDS; LKEM, NHLBI; PB, NCI; MN, NINDS; Division of Clinical Pharmacology, FDA

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Chemical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
_(a1) Minors
(a2) Interviews

X _(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Pharmacokinetic models are developed for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and humans. They provide a plausible set of equations that can be used to extrapolate data from animals to humans, and thereby improve chemotherapy and risk assessment. Consideration of regional drug delivery includes intra-arterial infusion, intracavitary administration, and direct intratumoral infusion. Work is well advanced on the development of a physiological pharmacokinetic model for methylmercury and inorganic mercury in the rat. Emphasis has been placed on the pharmacokinetics of macromolecules such as monoclonal antibodies and immunotoxins. Preclinical and Phase I pharmacology have been completed on immunotoxin injected into the CSF. Pharmacokinetics of alkylating agents used in cancer therapy provide a basis for correlating interspecies carcinogenicity.

RELEVANT BEIP PROJECTS: 73-159, 86-128, 89-108, 89-114, 89-116, 91-140, 92-122.

OBJECTIVES: To improve and extend mathematical models for the distribution and disposition of drugs, environmental contaminants, and endogenous metabolites in animals and humans to:

(1) Account for species differences in drug distribution.

(2) Provide a rational basis for extrapolating toxicity from experimental animals to humans.

(3) Provide a basis for optimizing cancer chemotherapy, in conjunction with pharmacodynamics.

(4) Enable rational transfer of *in vitro* thermodynamic and kinetic data to *in vivo* cases.

(5) Predict effective dose schedules of anticancer drugs in individual patients, with particular emphasis on regional drug delivery.

METHODS EMPLOYED: Mathematical models are developed from physicochemical, physiological, and anatomical information and the principles of chemical reaction engineering. Resulting ordinary or partial differential equations are solved analytically or numerically and compared with experimental data. Uncertainties are clarified by additional experiments and model modification.

(1) Work is well advanced on the development of a physiologically based pharmacokinetic model to describe the disposition of methylmercury and its major biotransformation product, mercuric mercury, in the growing rat. Model simulations are being tested against methyl- and mercuric mercury concentrations measured in rat tissues collected for a period of 98 days following methylmercury administration. To account for animal growth during the experimental period, the model includes time-dependent tissue volumes. Mercuric mercury is known to arise from demethylation of methylmercury, but it has always been unclear whether this biotransformation occurs in the body's tissues or as a result of microorganisms within the gastrointestinal tract. Model simulations now suggest that demethylation probably occurs to a significant extent at both sites. The model should be adaptable to the growing child, with appropriate choice of parameters.

(2) Increased emphasis has been placed on the pharmacokinetics of macromolecules such as diphtheria toxin (DT), immunotoxins, and monoclonal antibodies (MAbs). The experimental system uses human tumors grown in the flank of an athymic mouse. A physiologically based, compartmental pharmacokinetic model was used to determine tumor and normal tissue capillary permeability to these macromolecules, lymphatic loss, and apparent antigen concentration. Model-fitted values of antigen concentration were consistently lower than values calculated from *in vitro* and *ex*

vivo measurements--a possible indication that the distribution of the macromolecules within the tumor tissue is heterogeneous. This discrepancy motivated autoradiographic studies to examine the spatial distribution of DT and immunotoxins in tumors. Quantitative analyses were performed to calculate the fraction of the tumor's cross-sectional area that had attained a threshold concentration, and the kurtosis (a statistical measure of the dispersion in a distribution). The immunotoxin was distributed in punctate spots, whereas DT was distributed very uniformly. The maldistribution of the immunotoxin in the tumor, as compared to DT, may explain why DT is much more effective at inhibiting tumor growth than the immunotoxin, despite their equivalent toxicities to these cells in culture. Future studies are focused on strategies to attain greater homogeneity of the distribution of immunotoxins in tumors.

Chemical engineering theories on diffusion and reaction can be applied to the problem of macromolecular transport in tumors. Such theories predict that under circumstances of high antibody affinity or high antigen concentration, penetration of antibody into the tumor interstitium from the vasculature will be greatly retarded. The theories were recently applied to describe a two-step imaging or treatment protocol that uses a streptavidin-conjugated antibody (MAb-Av) as the first step, and radiolabeled biotin as the second step. These were compared to models for one-step application of directly radiolabeled antibody. The following predictions are based on the model analysis: (i) At equivalent molar doses of MAb-Av and MAb, distribution of radiolabel will be more heterogeneous for the two-step method. (ii) A two-step protocol permits an image to be obtained sooner after injection of radiolabeled material than does a one-step protocol. (iii) Optimization of a two-step protocol is strongly dependent on such properties as plasma kinetics of the antibody and the antigen turnover rate. Future modeling efforts will be aimed at incorporating details of the radiation dose distribution and the fate of an intracellular radiolabel.

(3) Intracavitary administration of macromolecules is being examined in the cerebrospinal fluid and in the peritoneal cavity.

For the treatment of leptomeningeal neoplasia, drugs can be administered directly into the cerebrospinal fluid (CSF). Clinical interest has been enhanced by the development of pharmacokinetic theory that predicts a large pharmacokinetic advantage in many cases. A Phase I clinical trial for a single dose of an immunotoxin injected into the ventricular CSF space has been completed. Clearance of the immunotoxin from the ventricles was characterized by an early phase half-life of 44 ± 21 min and a late phase half-life of 237 ± 86 min. The immunotoxin was cleared about 2.4 times more quickly than a coinjected extracellular marker, technicium-diethylene-triamepentaacetic acid (^{99m}Tc-DTPA). Possible explanations for the additional loss include binding to tumor cells or normal brain tissue or vessels, or transcapillary

loss. Acute toxicity at doses ≥ 120 μg consisted of transient headache, vomiting, and stupor with elevated intracranial pressure, which was responsive to steroids and CSF drainage. No systemic toxicity was detected. Preclinical studies in rats and monkeys revealed the importance of using a species-relevant antibody when testing an immunotoxin's toxicity; an immunotoxin made with an antibody targeted to the rat transferrin receptor was six times more toxic than an immunotoxin made with an antibody targeted to the human transferrin receptor. Moreover, the area under the concentration curve (AUC) of the rat-specific immunotoxin in the rat at the LD_{10} was a good predictor of the AUC at the maximally tolerated dose of the human-specific immunotoxin in humans. This finding also supports interspecies scaling concepts for pharmacokinetically-guided dose escalations. Pharmacokinetic findings from this trial will be applied to planning of a multiple-dose schedule for a PhaseI/Phase II clinical trial of another immunotoxin.

We are collaborating in studies examining the penetration of macromolecules from the blood or peritoneal cavity into normal tissues and human-tumor xenografts in the rat. Quantitative autoradiography was used to examine the tissue concentration profiles of IgG in tissues surrounding the peritoneal cavity following both intravenous and intraperitoneal administration. Following intravenous administration, tissue concentration profiles were relatively flat in most tissues and were not affected by dialysate osmolality. Following intraperitoneal administration, the tissue concentration profiles were significantly steeper, and concentrations increased with time (but were decreased by hypertonic dialysate). The hypertonic solution causes water flux into the peritoneal cavity, which dilutes the contents but does not prevent penetration of protein into the tissue. Since tissue uptake appears to be largely driven by hydrostatic pressure, movement against an apparent osmotically driven water flux suggests that the peritoneum functions as a heterogeneous structure which allows osmotically driven water transport into the cavity in some regions, with simultaneous convective movement from the cavity to the tissue in other regions.

(4) Dementia is commonly associated with acquired immunodeficiency syndrome (AIDS). While the human immunodeficiency virus (HIV) does not appear to be neurotropic, cells such as microglia within the brain may be infected, and this can apparently lead to central nervous system pathology. Two small clinical studies have suggested that administration of AZT directly into the cerebrospinal fluid (CSF) is safe; one of these reported some therapeutic effect. We are continuing to collaborate in the design of, and in the development of the rationale for, a clinical trial. Calculations suggest a very large and possibly exploitable pharmacokinetic advantage associated with direct administration of AZT into ventricular CSF, but AZT removal from the CSF is relatively rapid; ventricular levels, therefore, are probably not

representative of concentrations in the subarachnoid space. The depth of penetration of AZT into the brain from the CSF is a critical issue that we are addressing in rats both experimentally and theoretically by means of microdialysis.

SIGNIFICANCE: Drugs and other chemicals are tested for effects in animals and *in vitro* systems, with the aim of extrapolating the results to humans. Both the risk associated with environmental contaminants and the optimization of therapy are at issue.

PROPOSED COURSE: To continue pharmacokinetic modeling, with consideration of pharmacodynamic and cytokinetic events and drug interactions. To continue the support of regional procedures and other measures to overcome drug resistance. To perform research designed to investigate distribution and metabolism of environmental contaminants, and to find methods for incorporating pharmacokinetics in models of risk assessment. To investigate chemical metabolism *in vitro*, in conjunction with pharmacokinetic models for quantitative prediction of metabolism *in vivo*. To extend distributed models for the description of drug movement through tissue. To study the transport of macromolecules.

PUBLICATIONS:

Shockley TR, Lin K, Sung C, Nagy JA, Tompkins RG, Dedrick RL, Dvorak HF, Yarmush ML. A quantitative analysis of tumor-specific monoclonal antibody uptake by human melanoma xenografts: effects of antibody immunological properties and tumor antigen expression levels. Cancer Res 1992;52:357-66.

Sung C, Shockley TR, Morrison PF, Dvorak HF, Yarmush ML, Dedrick RL. Predicted and observed effects of antibody affinity and antigen density on monoclonal antibody uptake in solid tumors. Cancer Res 1992;52:377-84.

Flessner MF, Dedrick RL, Reynolds JC. Bidirectional peritoneal transport of immunoglobulin in rats: compartmental kinetics. Am J Physiol 1992;262:F275-87.

King FG, Dedrick RL. Physiological pharmacokinetic parameters for cis-dichlorodiammineplatinum(II) (DDP) in the mouse. J Pharmacokin Biopharm 1992;20:95-9.

Dedrick RL, Morrison PF. Carcinogenic potency of alkylating agents in rodents and humans. Cancer Res 1992;52:2464-7.

Flessner MF, Dedrick RL, Reynolds JC. Bidirectional peritoneal transport of immunoglobulin in rats: tissue concentration profiles. Am J Physiol (in press).

Sung C, Wilson D, Youle RJ. Comparison of protein synthesis inhibition kinetics and cell killing induced by immunotoxins. J Biol Chem 1991;2.66:14159-62.

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NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10034-15 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Three-Dimensional Histological Reconstruction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Stephen Leighton, Sc.D. Mechanical Engineer BEIP, NCRR

COOPERATING UNITS *(If any)*
Marine Biological Laboratory (A. Kuzirian); NSS, NINDS (J. Olds)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A semiautomatic system for acquiring three-dimensional structural information about histological material is being developed. The system should be faster and more reliable than techniques that use serial sections, although resolution may be limited. In brief, an embedded tissue block will be fixed relative to a scanning electron microscope imaging system; the surface of the block will be imaged and the image stored, and successive slices will be removed by a built-in microtome. Handling and registration of thin sections will thus be eliminated. Human and computer pattern recognition will transform the resulting set of images into a three-dimensional reconstruction. The images of *Hermissenda crassicornis* obtained by this technique correlate well with TEM images of the same tissue.

OBJECTIVES: (1) To facilitate making schematic diagrams of neural networks. (2) To facilitate developmental studies of small organs and organisms. (3) To do three-dimensional reconstruction of biological structures.

METHODS EMPLOYED: Preliminary experiments were made with specimens embedded in media other than water, then frozen. Manual cutting was also tried on these frozen specimens.

MAJOR FINDINGS: A variety of nonaqueous embedding materials are candidates for cryoembedding and freeze-etching. They exhibit desirable mechanical properties for cryomicrotomy and reasonable behavior during freezing, as well as reasonable etch rates. As opposed to water, these materials do not expand appreciably during freezing; morphology is thus well preserved.

PROPOSED COURSE: The best of these candidate materials will be tried in the SEM and in an actual cryomicrotome.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10039-15 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biophysical Instrumentation and Methodology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Marc S. Lewis, Ph.D. Research Chemist BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Microanalysis Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The project is intended to develop new instrumentation and methods, and to improve existing instrumentation and techniques, for the characterization of biological macromolecules and the study of their interactions. Analytical ultracentrifugation, the techniques ancillary to it, and methods of data analysis using mathematical modeling appropriate for these techniques are the major areas of interest.

Studies have continued on the application of mathematical modeling to problems of ultracentrifugal analysis, and on the development of new methods of performing experiments and analyzing data. In particular: (1) Application of the method of implicit constraints to the analysis of simultaneous homogeneous and heterogeneous associations has been achieved. (2) A new method for the analysis of sedimentation velocity experiments has been developed.

OBJECTIVES: To develop data acquisition systems for analytical ultracentrifuges and ancillary equipment, such as plate or film readers and densitometers; to develop appropriate software for use with the acquisition systems; to develop new instrumentation to enhance the sensitivity of analytical ultracentrifuges; to study means of using MLAB software more effectively for the analysis of ultracentrifuge data by mathematical modeling techniques; and to develop new software for this purpose that can be used on the Macintosh II and similar computers.

METHODS EMPLOYED:
Mathematical modeling studies have involved the use of computer simulations, as well as the analysis of experimental data. The objective of the method of implicit constraints is to write a mathematical model to be used for data analysis in such a form that the minimum number of parameters will be used, all parameters will be constrained to real and physically meaningful values, and the sum-of-squares surface will have an optimal shape with respect to the values of the fitting parameters. This approach has been particularly successful for a variety of interacting systems. In particular, it has been applied with considerable success to the analysis of both the homogeneous association of the P66 subunits and the heterogeneous association of the P66 and P51 subunits of HIV reverse transcriptase. Because this method involves the assumption of conservation of mass within the centrifuge cell, it was found necessary to develop methods for validating this assumption.

A new equation has been derived for fitting concentration distributions obtained in sedimentation velocity experiments by simultaneously computing the radial position of the second moment of the mass of the solute molecular species and the width of the gradient boundary, thus permitting determination of both the sedimentation coefficient and the diffusion coefficient of the solute species in a single experiment. This method has found application in several studies where it was advantageous to use the values of the molecular mass and the sedimentation coefficient to calculate the axial ratio of the molecule being studied.

MAJOR FINDINGS: The successful application of the method of implicit constraints to the study of the interactions of the P66 and P51 subunits of HIV reverse transcriptase, and its application to the interaction of nucleic acids with DNA polymerase-β, are discussed in project report 10184-09. The publication describing the methodology is listed below; the publication on the role of the interaction is listed in the other report.

An equation has been derived which, when used as a mathematical model, fits well the concentration distribution obtained during a sedimentation velocity experiment of one or two macromolecular species. One of the parameters in this model gives the radial position of the second moment of the mass in the sedimenting boundary; the time dependence of this radial position gives the

sedimentation coefficient. The time dependence of the parameter that governs the width of the gradient permits calculating the apparent diffusion coefficient. These parameters can be obtained for two components, with some loss of precision, even if they are only moderately separated during the experiment. Because the sedimentation and diffusion coefficients can be used to calculate the molecular weight, this quantity can now be obtained in a very rapid experiment. The publication describing this method is now in press.

The use of the new analog-to-digital converter systems with different computers has already enhanced data acquisition and analysis significantly for both equilibrium and velocity experiments. Data of markedly enhanced quality and quantity are readily obtained, and this improvement has led to success with more sophisticated and more difficult experiments than had been possible previously. The PC version of MLAB has greatly facilitated data analysis in these studies, and has been of great use for simulation studies performed in this laboratory; it is the standard used for evaluating other possible systems. A publication describing methods of data acquisition and analysis is in press.

SIGNIFICANCE: Improved experimental methods, improved methods of data acquisition, and improved methods of data analysis by mathematical modeling all contribute significantly to the quality and cost-effectiveness of research involving analytical ultracentrifugation. The development of these methods and the improvements in ultracentrifugal instrumentation will greatly facilitate such studies, and will free the investigator from the constraints of obsolete instrumentation.

PROPOSED COURSE: To continue studies of the types described above.

PUBLICATIONS: Lewis MS. Ultracentrifugal analysis of a mixed association. Biochemistry 1991;30:11716-19.

Attri AK, Lewis MS. A fitting function for the analysis of sedimentation velocity concentration distributions. In: Harding SE, Rowe A, eds. Analytical ultracentrifugation in biochemistry and polymer science. Cambridge: Royal Society of Chemistry, 1992 (in press).

Lewis MS. Data acquisition and analysis systems for the absorption optical system of the analytical ultracentrifuge. In: Harding SE, Rowe A, eds. Analytical ultracentrifugation in biochemistry and polymer science. Cambridge: Royal Society of Chemistry, 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10097-12 BEI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Studies in Cardiovascular Dynamics

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

Richard Chadwick, Ph.D.	Head, Theor. Biomech. Group	BEIP, NCRR
Cheng Dong, Ph.D.	Staff Fellow	BEIP, NCRR

COOPERATING UNITS *(if any)*

INSERM U. 120 (A. Azancot-Benisty); INSERM U. 141 (B. Levy); Laboratory of Physical Mechanics, Univ. of Paris XII (J. Ohayon, C. Oddou)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Office of the Director

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We have refined our existing model for myocardial and ventricular mechanics to include the effects of collagen on the elasticity of the myocardium and filling of the left ventricle; wall thickness and curvature effects; pathological fiber organization; myocardial blood flow; myocardial oxygen demand; effects of electrical activation patterns; interaction with the right ventricle; and dynamic vibrations. The model was used in conjunction with ultrasonic kinematic data, gated radionuclide ventriculography, and left-heart catheterization data; and implanted ultrasonic crystals were used to determine performance and contractility parameters. The physiological relationships between phasic coronary arterial, venous, and left ventricular pressure and blood flow were determined during a long diastole and control conditions. A theoretical model was developed to describe contraction of arterioles, and will be used to study mechanisms of hypertension. Theoretical models investigating residual stress in the myocardium and contraction with an ischemic zone are being developed. Congenital heart defects with right ventricular pressure and/or volume overload have been modeled. The hypothesis of independence of mean circumferential shortening velocity on preload is being investigated via the model and echocardiographic patient data.

OBJECTIVES: To provide a framework for determining ventricular function, both at the fiber level and for the entire structure; to relate the relationship between coronary pressure and flow to the poroelastic rheology of the myocardium; to provide a framework for evaluating those aspects of cardiovascular function that depend on the filling of, and ejection from, the left ventricle; to develop new noninvasive measurement techniques for cardiovascular research; and to provide a theoretical framework for the prediction of myocardial ischemia and the severity of congenital heart defects.

METHODS EMPLOYED: An interdisciplinary approach is used involving clinical ultrasound data, hemodynamic measurements on animals, physical models, and mathematical models.

SIGNIFICANCE: This research program, with its interdisciplinary approach, has the potential to increase fundamental knowledge in cardiovascular physiology and to improve diagnosis and treatment of cardiovascular disease.

PROPOSED COURSE: Experiments are planned with INSERM U. 141 to investigate the effects of vessel stretch by volume loading on coronary blood flow. The theoretical model will be extended to include this effect. Experiments are also being planned to determine the mechanical properties of arterioles as a function of muscle tone and transmural pressure, and interpretation will be made with the theoretical model. The theoretical model of the left ventricle is being extended to deal with regional abnormalities. This necessitates developing mathematical techniques to deal with nonaxisymmetric conditions. Experiments are being carried out at the University of Paris to assess the poroelastic properties and residual stress in the myocardium.

PUBLICATIONS: Ohayon J, Chadwick RS, Azancot-Benisty A. Influence du ventricule droit sur la mécanique du ventricule gauche. In: Proceedings of the 10^{e} Congrès Français de Mécanique. Paris: 1991 (in press).

Ohayon J, Tedgui A, Chadwick RS, Oddou C. Mechanics of the artery and microstructure of the wall. International Union of Theoretical and Applied Mechanics 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10098-12 BEI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Laser Instrumentation for Vitreous and Cardiovascular Microsurgery

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert Bonner, Ph.D.	Physicist	BEIP, NCRR
Philippe Douek, M.D.	Guest Researcher	BEIP, NCRR

COOPERATING UNITS *(if any)*

Clinical Branch, NEI (C. Kupfer); Optical Science Branch, Naval Research Laboratory; Cardiology Branch, NHLBI (S. Epstein); Quantronix Corporation; Spectraphos; Intratherapy, Inc.; Washington Cardiology Center

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects _(b) Human tissues (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This program initially developed laser microsurgical techniques centered on ophthalmological applications (pulsed carbon dioxide lasers and pulsed ND:YAG slit lamp-based laser systems). Its primary focus for the last seven years, however, has been in cardiological applications, in particular laser angioplasty and the development of pulsed, solid-state infrared lasers for microsurgery. We demonstrated the feasibility of transmission through flexible fiber optics of pulsed infrared, visible, and ultraviolet lasers, and the tissue responses to these lasers. In particular, we have measured the dependence of acoustic transients and ablation efficiency on increasing pulse fluence with catheter systems used clinically, and have concluded that acoustic transients play a dominant role in the clinical results of pulsed laser angioplasty. In conjunction with the Naval Research Laboratory and Quantronix Corporation, new infrared laser sources were developed which utilize strong water absorption and can be transmitted through low-loss, cladded optical fibers. Clinical trials of peripheral and coronary laser angioplasty were completed, using either pulsed-dye or pulsed infrared lasers with computer-controlled fluorescence guidance. Recent work has involved identification of the tissue and biological effects of pulsed lasers--in particular, the large role that acoustic transients and transient, moderate (~60°C) thermal elevations play in acute and chronic clinical responses. Principally, since pulsed laser angioplasty mechanically disrupts the atheroma at stenoses rather than ablating or removing it, optimization of clinical results must be balanced against increased rates of dissection.

PHS 6040 (Rev. 5/92)

OBJECTIVES: Current objectives are: (1) to evaluate the relative advantages of various pulsed laser sources for angioplasty (pulsed-dye, excimer, and Tm:YAG lasers); (2) to design and develop microsurgical systems using these sources; (3) to determine the proper methodology for using these laser systems; (4) to evaluate the performance of these systems both *in vitro* and *in vivo*, and in comparison with new, alternative nonlaser techniques; and (5) to examine other areas of clinical importance when using these devices, in particular the response of tissues to acoustic and thermal injury (e.g., restenosis).

METHODS EMPLOYED: Careful dosimetry of laser-tissue effects determines relative efficacy of the different laser sources. Tissue histological damage, as well as thermal transients surrounding the ablated volume, are quantified. Variation in laser pulse parameters (wavelength, temporal structure, and spatial structure) are performed in order to characterize the physical processes involved, and to understand and predict effects. A variety of physical detection methods have been used in order to evaluate effects and to test models of laser damage in tissue, both *in vitro* and *in vivo*. Final evaluation of laser system performance is based on intravascular ultrasound examination of clinical lesions before and after therapy, in conjunction with long-term follow-up.

MAJOR FINDINGS: (1) Pulsed dye-laser angioplasty was able to cross total occlusions in patients, principally by the disruptive force of acoustic transients associated with tissue and blood vaporization. (2) Blood-enhanced local absorption on/within tissue allowed success of multifiber catheters at pulse fluences below the ablation threshold observed in the absence of blood. Fluorescence spectroscopy during laser sequences confirmed the presence of thin blood layers >50μm between ~70% of the optical fibers and the tissue. (3) Associated with the large acoustic transients generated by rapid expansion of vapor bubbles was a high incidence of coronary artery dissections. (4) Laser-induced shock waves can pace ventricular contraction (although ECG-gating of laser pulses to the ventricular refractory period or high pulse repetition rates can inhibit the abnormal pacing). (5) Acoustic transients appear to tear the annuli of stiff plaque associated with stenoses, thereby overcoming their constraint of vessel lumen and allowing lumen expansion. However, this mode of action appears to be intrinsically prone to a high rate of dissection, and is only associated with minimal debulking of stenotic lesions. Pulsed laser angioplasty, in our studies and in others, has been generally unsuccessful in very stiff, highly calcified stenoses. (6) New "smooth pulse" Tm:YAG (or Er:YAG) laser systems that we developed with the Naval Research Laboratory can ablate with smaller acoustic transients and may reduce the dissection rate, but their efficacy in angioplasty might also be limited.

These ambitious laser coronary angioplasty systems might provide possibilities for advancements in microsurgery in a variety of other fields.

SIGNIFICANCE: Successful development of an efficacious means of relieving atherosclerotic obstructions in human blood vessels (particularly the coronary arteries) by percutaneous laser angioplasty could have a dramatic effect on the practice of cardiology and vascular surgery, if the clinical results could be improved to the point of being superior to those of balloon angioplasty or other, newer mechanical atherectomy devices. The Tm:YAG laser/silica optical fiber systems and Er:YAG laser/fluoride fiber that we have worked on appear to have numerous applications in other areas of endoscopic microsurgery.

PROPOSED COURSE: Refinement of current prototype laser microsurgery systems; testing of their applicability to laser angioplasty, ophthalmic microsurgery, and ablation of bone through *in vivo* and *in vitro* studies, with evaluation of efficacy in clinical trials.

PUBLICATIONS:

Bartorelli AL, Leon MB, Almagor Y, Prevosti LG, Swain JA, McIntosh CL, Neville RF, House MD, Bonner RF. *In vivo* human atherosclerotic plaque recognition by laser-excited fluorescence spectroscopy. J Amer Coll Cardiol 1991;6(suppl B):160B-168B.

Lawrence JB, Prevosti LG, Kramer WS, Smith PD, Bonner RF, Lu DY, Leon MB. Pulsed laser and thermal ablation of atherosclerotic plaque: morphometrically defined surface thrombogenicity in studies using an annular perfusion chamber. J Amer Coll Cardiol 1992;19:1091-1100.

Douek P, Bonner RF. Fluorescence-guided pulsed dye laser-assisted angioplasty. Radiology 1992;182:897L.

Douek PC, Correa R, Neville R, Unger EF, Shou M, Banai S, Ferrans VJ, Epstein SE, Leon MB, Bonner RF. Dose-dependent smooth muscle cell proliferation induced by thermal injury with pulsed infrared lasers. Circulation 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10109-12 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Adjunctive Heat Treatment of Cancer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

Ronald L. Levin, Sc.D.	Biomedical Engineer	BEIP, NCRR
James Mattiello, Ph.D.	Staff Fellow	BEIP, NCRR
Ching-Nien Chen, Ph.D.	Physical Scientist	BEIP, NCRR

COOPERATING UNITS *(if any)*
DR, CC (D. LeBihan); LCE, NHLBI (R. Turner)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
  _(a1) Minors
  (a2) Interviews
_(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to develop adjunctive hyperthermia modalities for cancer treatment through theoretical and experimental studies of the spatial and temporal variation in the temperature field of tissues subjected to radio-frequency electromagnetic radiation. We are currently involved in measuring the patterns of energy deposition produced by a miniannular phased array (MAPA) applicator within various types of extremity phantoms, describing the electromagnetic fields of a MAPA in terms of its design parameters, describing the transient thermal profiles within limbs produced by a MAPA, and describing the systemic temperature and cardiac changes associated with heating various regions of the body. We are also continuing to refine a noninvasive method for measuring temperature *in vivo*, using magnetic resonance imaging of molecular diffusion.

OBJECTIVES: To optimize the energy deposition patterns of hyperthermia applicators; to continue to develop a generalized mathematical model that will predict the spatial and temporal variation of the temperature field within a tissue or organ being heated; to continue to develop a noninvasive method for monitoring temperature during hyperthermia; and to develop optimal adjunctive hyperthermia modalities.

METHODS EMPLOYED: Experimental measurements of the resulting thermal profiles within phantoms and animals being heated by our hyperthermia applicators will be accomplished through the use of a dedicated PC/AT computing system, its associated data acquisition system, a BSD-200 thermal monitoring subsystem, a Luxtron 3000 thermal monitoring subsystem, and a GE OMEGA 4.7-T NMR unit.

MAJOR FINDINGS: Temperature images are obtained noninvasively, with high accuracy (0.2°C resolution, using 2-min scans), using images of molecular diffusion. Temperature MR images agree very well with temperature measurements obtained from fiber-optic probes placed inside gel phantoms and animals. The temperature resolution was found to be 0.2°C, with a spatial resolution of 1 cm.

SIGNIFICANCE: At present, the heat treatment of cancerous cells shows considerable promise in the management of cancer, when combined with conventional radiotherapy and chemotherapy; nevertheless, there still remain numerous important problems that must be resolved. Of paramount importance is the problem of generating and controlling uniform temperature fields within tissues. This study will therefore attempt to facilitate the development of optimum hyperthermia modalities by theoretically and experimentally studying the temperature fields within tissues during hyperthermia.

PROPOSED COURSE: Nonclinical *in vivo* work is continuing, using the GE 4.7-T Omega unit for studying the temperature dependence of diffusion during hyperthermia, and the GE 1.5-T Signa for development of real-time MRI temperature-monitoring methods, using echo-planar imaging techniques.

PUBLICATIONS: Charny CK, Levin RL, Weinbaum S. Simulations of heat transfer in an axisymmetric layer of perfused muscle using a modified three-equation model. Trans ASME J Biomechanical Eng 1991 (in press).

Zhang Y, Samulski TV, Joines WT, Mattiello J, Levin RL, LeBihan D. On the accuracy of noninvasive thermometry using molecular diffusion magnetic resonance imaging. Intl J Hyper 1992;8:263-74.

AWARDS: The 1991 Sylvia Sorkin Greenfield Award, given by the American Association of Physicists in Medicine for the best nondosimetry paper published in Medical Physics during 1990, was awarded for the paper "Hyperthermia System Combined with a MRI Unit" (1990;17:855-60), authored by J. Delannoy, D. LeBihan, D. I. Hoult, and R. L. Levin.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10112-12 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Analysis of Microcirculatory Blood Flow by Laser Doppler Scattering

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Robert F. Bonner, Ph.D. Physicist BEIP, NCRR

COOPERATING UNITS *(if any)*
HB, NHLBI; CB, NEI; LCI, NIAID; SN, NINDS; LAP, DCRT (R. Nossal); TSI, Inc., St. Paul, MN

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
X _(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project has developed a clinically useful method (laser Doppler instrument and its theory of operation) for clinical measurements of microcirculatory blood flow, the density of flowing red blood cells (RBCs), and mean RBC velocity. Assistance has been given to the commercialization of this methodology and its application to clinical research. Our clinical studies have been directed toward examining normal and abnormal microvascular dynamics through noninvasive clinical studies of skin and nasal mucosa, and toward intraoperative clinical studies of muscle and the CNS. Considerable theoretical work has been directed toward an adequate construct with which to interpret the physical measurements, and to refining the accuracy of the microcirculatory measurements. We have discovered abnormal microcirculatory patterns and responses in the skin of patients with sickle-cell disease, hypertension, certain cardiac circulatory syndromes, diabetes, and skin cancer. The microcirculatory effects of therapy are monitored with this technique, affording a better understanding of the microcirculatory components of these diseases.

OBJECTIVES: To develop our understanding of the human microcirculation in health and disease, and to refine the clinical utility of laser Doppler measurements of the microcirculation.

METHODS EMPLOYED: Application of laser Doppler flow meters to various clinical protocols is based on the understanding of this technique and of the microcirculation of the targeted tissue. As very little clinical work has heretofore been performed on microcirculatory dynamics, protocols are frequently designed to detect significant abnormalities and then to characterize them. Theoretical modeling of the signal and, to a limited degree, of the microcirculation, facilitates the interpretation of the microcirculatory measurements.

MAJOR FINDINGS: (1) The blood volume and mean RBC velocity signals, as well as the blood flow signals obtained by the laser Doppler method developed at the NIH, provide reproducible and clinically useful measurements. (2) A light-diffusion theory and empirical optical measurements have better defined the sample volume and the absolute calibration of the measurements for a variety of tissues. (3) Myogenic vasomotion in a variety of human tissue can be detected and quantified easily. This activity is dramatically increased in a variety of diseases, and may play a critical role in microcirculatory adaptation to microvascular or rheological abnormalities. (4) Reliable measurements in the central nervous systems (CNS) of patients undergoing surgery can be made with handheld probes, and appear useful in analyzing the local microvascular response during AVM surgery. (5) Skin blood flow measurements can be used to quantify sympathetic innervation in patients with surgical sympathectomy or neuropathy. (6) The measurement of microcirculatory response to photodynamic therapy of tumors has been evaluated, both during and following therapy. (7) Studies of the peripheral microcirculation in diabetics have identified a functional loss of maximal microcirculatory dilation, which may have an important role in the morbidity associated with impairment and loss of limbs in long-term diabetics.

PROPOSED COURSE: To pursue clinical protocols in order to establish and evaluate the contribution of microcirculatory abnormalities to a variety of diseases. To refine the technology in order to facilitate laser Doppler measurements in a variety of tissues, and in particular, those accessible via endoscopy. To continue to assist commercial manufacturers to develop accurate, reliable, and useful instruments based upon this technology.

SIGNIFICANCE: The clinical research studies performed in this project have provided new means of quantifying the microvascular changes (in particular, microvascular dynamics) of a variety of diseases, such as sickle-cell disease, hypertension, scleroderma, diabetes, and allergy, as well as microvascular changes both during and following surgical and nonsurgical therapies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10122-11 BEI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Microcomputer Applications for the NIH Biotechnology Unit (Pilot Plant)

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas R. Clem, Sr., BSEE Electronics Engineer EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LCDB, NIDDK (Y. Shiloach); Fluor Daniel, Inc. (J. Hsiao)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.25	1.0	0.25

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project is concerned with satisfying the instrumentation needs of the NIH Biotechnology Unit (Pilot Plant) using modern equipment and low-cost desktop- or laptop-sized personal computers (PCs). PCs have been acquired and installed to supply a variety of services, including process control, data acquisition, data analysis, and administrative functions. The instrumentation and process control functions use commercial equipment as much as possible, and are interfaced using standard I/O connections, such as the IEEE-488 GPIB and the RS-232 Serial I/O ports, as well as direct digital and analog I/O components in the desktop computer.

A standard data acquisition and control program has been written and is being adapted for each fermentor system in the Biotechnology Unit. By providing the same operator interface for each system, users need to learn only one command structure to be able to operate any system. Using these techniques allows changes in the parameters measured or controlled to be accomplished relatively quickly and easily. Utilizing the computational capabilities of the computer/controller allows initial selection of the operating parameters and dynamic alteration of these parameters as the process continues, thus allowing optimization of yields or detailed study of the process parameters. Adaptive control techniques have been applied to the process, and further increases in product yield are being realized.

OBJECTIVE: To increase efficiency and productivity of the Biotechnology Unit, to expand the fermentation process capability, and to develop methods for researching the fermentation process through the expanded use of inexpensive desktop personal computers and more sophisticated control and data acquisition programs.

METHODS EMPLOYED: Desktop personal computers and interface electronics, both commercial and custom-designed, are connected to laboratory instruments and to fermentation vessels via the IEEE-488 GPIB, via the RS-232, or by direct connections. Interfacing techniques are kept as versatile as practical to allow system changes to be accomplished relatively easily. The control programs are written in the high-level language Pascal so that program changes can be made easily. All data are stored in a general form (ASCII) that most data analysis programs can access. A CRADA, "Development of an Adaptive Control System for the Fermentation Process" (number DK0009), was continued this year. This collaboration has involved the extension of the data acquisition and control programs to include adaptive control, which provides much better control of selected process parameters, such as dissolved oxygen, with a resultant increase in product yield.

SIGNIFICANCE: Computer monitoring and control of the fermentation process provide several significant advantages over manual methods. By using the computer to make decisions based on what is occurring in the fermentation process, parameters can automatically be altered to increase yields of a product or to produce it in purer form. The computer can also perform some of the "housekeeping" tasks associated with running a fermentation process that would normally occupy an operator. Complete records of any run are saved on disk storage for later analysis. Computerized data acquisition and control allow growth rate and other parameters to be assessed under a variety of environmental conditions. The presence of a computer in the Biotechnology Unit office provides administrative support in the form of recordkeeping and text editing. This support has taken on new importance in the light of recent concern over safety and requirements for detailed logs of materials grown in the Pilot Plant.

PROPOSED COURSE: To expand the system to monitor and control additional parameters. (Larger fermentation vessels with more modern control systems, in the process of being acquired by the pilot plant, will be interfaced with our current systems.) To apply knowledge gained from previous work to new systems being assembled in support of the new Protein Expression Laboratory. To apply adaptive control capabilities to the other systems in the Biotechnology Unit and the Protein Expression Laboratory. To investigate the relative merits of other growth control techniques, such as maintaining dissolved oxygen level by feeding nutrient based on changes in measured DO_2 level.

PUBLICATIONS: Andorn N, Kaufman JB, Clem TR, Fass R, Shiloach J. Large-scale growth of *Bordetella pertussis* for production of extracellular toxin. In: Goldstein WE, Dibiasio D, Pedersen H, eds. Biochemical engineering VI: annals of the New York Academy of Sciences. New York: New York Academy of Sciences, 1990;589:363-71.

Shiloach Y, van de Walle, Kaufman JB, Clem TR, Fass R. Bacterial fermentation for the production of native and recombinant bacterial protein toxins. In: Yu PL, ed. Fermentation technologies: industrial applications. London: Elsevier Applied Science, 1990;79-84.

Hsiao J, Ahluwalia M, Kaufman J, Clem T, Shiloach J. An adaptive control strategy for maintaining dissolved oxygen concentration in high density growth of recombinant *E. coli*. In: Biochemical engineering VII: cellular and reactor engineering. New York: New York Academy of Sciences, 1991 (in press).

Hsiao J, Ahluwalia M, Kaufman JB, Clem TR, Shiloach J. Use of an adaptive control strategy for the production of Exotoxin A from high density culture of recombinant *Escherichia coli*. In: Furusaki S, Endo I, Matsuno R, eds. Biochemical engineering for 2001: proceedings of Asia-Pacific biochemical engineering conference 1992. Tokyo: Springer-Verlag, 1992;150-3.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10162-10 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Wound Healing: Biology and Rheology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas L. Talbot, M.S. Mechanical Engineer BEIP, NCRR

COOPERATING UNITS *(if any)*
SB, DCT, NCI (J. Norton, G. Salomon); ROB, DCT, NCI (W. De Graff)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Preliminary studies have been completed with swine models. These studies involved stamping an indelible grid (10 cm x 10 cm) on the skin of an anesthetized swine, excision of a 1 cm x 10 cm strip of the skin out of the grid area, and approximating the incision edges with silk sutures. Photographs of the grid were taken before excision, after excision, and after suturing. These photographs are being analyzed to determine the impressed strain on the wound closure, and eventually to relate this information to wound breaking strength (WBS).

Studies based on an anesthetized rat model that relates biological and pharmacological interventions to WBS have been completed. Certain groups were treated pharmacologically during the wound-healing process. Significant decrease in WBS was observed in these groups, compared to control groups. Further studies will include the comparison of a tumor-bearing group to control groups.

MAJOR FINDINGS: Rats treated with 8 mg/kg Adriamycin prior to, or on the day of, wounding demonstrated decreased wound breaking strength in incisional wounds at all intervals after wounding.

Decreased amounts of collagen and DNA, lower mitotic rates, and decreased cellularity were noted in wound chambers from rats treated in this manner. In both the incisional wound and wound chamber models, rats treated with Adriamycin seven days after wounding showed a less dramatic healing impairment. No differences in collagen type were noted between chambers from the Adriamycin-treated rats and those from untreated rats. The data suggest deficient quantities of platelet, macrophage, and lymphocyte factors; a decreased rate of collagen synthesis; and impaired collagen remodeling. Wound healing was examined in two wound healing models in control rats and animals bearing methylcholanthrene-induced sarcomas, in order to evaluate experimentally the effects of tumors on wound breaking strength. In a dorsal incisional wound model, a significant decrease in wound breaking strength was noted from 19 days after the implantation of tumor onward. The amount of the breaking strength deficit progressed with the size of the tumor. In a wound chamber model, hydroxyproline levels were measured as an index of collagen content, and 3 H-thymidine incorporation was related to DNA content in order to give an index of DNA synthesis. Histology and collagen types were also examined in the wound chamber model. The tumor-bearing state produced no significant change in any of the parameters studied in the wound chambers. The tumor-bearing state appears to inhibit wound healing in skin wounds, but has no effect on healing in a model of a deeper wound. This difference in healing in the two wound models may be explained by a crosslinking deficiency, or by differential capacities for healing different types of wounds in tumor-bearing rats.

TGF-beta, which is now a readily available growth factor as a result of recombinant techniques, has been reported as a wound healing enhancement agent. A series of experiments were run that incorporated our present wound model, to investigate the effects of various concentrations of TGF-beta at the wound site. Paired dorsal incisions were filled with an absorbable collagen matrix, to which either 0.5, 5, or 50 ug of TGF-beta was added. Preliminary results indicate that TGF-beta does indeed affect wound healing, as assessed by wound breaking strength. However, depending on the dosage, the wound healing effect may be an increased or decreased resistance to wound bursting.

Similar experiments were performed using guinea pigs as the experimental model, for straightforward comparison of results from previously published reports demonstrating either wound healing enhancement or deterioration.

PROPOSED COURSE: More experiments are underway that will focus primarily on determining the best dosage of TGF-beta to produce optimal wound breaking strength.

PUBLICATIONS: Bernstein EF, Harisiadis L, Salomon G, Talbot TL, et al. Transforming growth factor-beta improves healing of radiation-impaired wound. J Invest Derm 1991;97:430-4.

Salomon GD, Kasid A, Cromack DT, Director E, Talbot TL, Sank A, Norton JA. The local effects of cachectin/tumor necrosis factor on wound healing. Ann Surg 1991;214:175-80.

Salomon GD, Kasid A, Director E, Talbot TL, Sank A, Norton JA. The effects of local tumor necrosis factor on wound healing. Surg Forum 1989;40:637-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10184-09 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Physical Chemistry of Biological Macromolecules

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Marc S. Lewis, Ph.D. Research Chemist BEIP, NCRR

COOPERATING UNITS *(if any)*
Ctr. for Mol. Sc., Univ. of Texas Med. Branch (S. Wilson); LB, NCI (C. Vinson); LCB, NIDCD (S. Yoo)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Microanalysis Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.7	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects X _(b) Human tissues (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to study the physical properties of a wide variety of biological macromolecules, with the goal of correlating these properties to the structure and function of the macromolecules. The emphasis is on the thermodynamics of the interactions of these macromolecules and on their molecular size and shape. Analytical ultracentrifugation and mathematical modeling are the principal research techniques used.

Studies on HIV reverse transcriptase have been directed toward investigating the thermodynamics of the homogeneous association of the P66 subunit, and the heterogeneous association of P66 with the P51 subunit. Studies on chromagranin A have been directed toward investigating the effects of pH and calcium ion concentration on the thermodynamics of the aggregative properties of the molecule, and toward studying these same factors for various peptide domains of the molecule. Studies on DNA polymerase-b have been directed toward investigating the conformational properties of the molecule by fluorescence and ultracentrifugal studies, and toward investigating the interaction of the enzyme with nucleic acids. Studies on CEB-63, a leucine-zipper peptide, have been directed toward determining the thermodynamic parameters characterizing its reversible aggregation.

METHODS EMPLOYED: The macromolecules have been isolated and purified from appropriate sources by conventional methods. The associations have been studied by establishing equilibrium gradients in the analytical ultracentrifuge, then analyzing the resultant data by means of mathematical modeling techniques using PC MLAB.

MAJOR FINDINGS: HIV Reverse Transcriptase: HIV reverse transcriptase is an enzyme involved in the replication of HIV, and is composed of two subunits, P66 and P51. It has been postulated, but never clearly demonstrated, that P66 undergoes dimerization, and also interacts with P51. Using the method of implicit constraints developed in this laboratory, it has been definitely established that P66 forms a reversible dimer with a free energy change of -5.99 kcal/mole, and that P66 reversibly interacts with P51 with a free energy change of -7.24 kcal/mole. The difference of 1.25 kcal/mole indicates that the equilibrium constant for the P66-P51 interaction is approximately ten times greater than the equilibrium constant for the P66-dimer formation. This work has been published. Additional work in this area has been directed toward a complete thermodynamic characterization of the P66 dimerization and the P51-P66 interaction. The experimental work has been completed, and the data is being analyzed.

Chromagranin A: This major protein, found in the secretory vesicles of the adrenal medulla, is involved in both the storage of epinephrine and norepinephrine, and the storage and release of calcium in chromaffin cells. These studies have shown that the protein is involved in a reversible monomer-dimer reaction at pH 7.5, and a monomer-tetramer reaction at pH 5.5. Low calcium concentrations slightly diminish aggregation, but physiological concentrations appear to increase both dimer and tetramer formation above the levels observed in the absence of calcium. This work has been published. Additional work in this area has been a study of the associative properties of various peptide domains of the chromagranin A molecule. A peptide has been isolated and studied that comprises approximately one-tenth of the intact molecule and has the same pH and calcium ion aggregation dependency as the whole molecule. The differences between the thermodynamic parameters characterizing its interactions and those of the whole molecule indicate the role of the remainder of the molecule in influencing the interactions.

DNA polymerase-β: Structural studies on this DNA repair enzyme demonstrate that it has an approximately 5-to-1 axial ratio for the intact molecule, a 2-to-1 axial ratio for the small (8-kD) domain involved in single-strand DNA binding, and a 5.5-to-1 axial ratio for the larger (31-kD) domain that binds double-stranded DNA. These data indicate that the 8-kD domain is folded back on the 31-kD domain. Studies on the binding of a single-strand DNA to both the 8-kD domain and the intact molecule show much stronger binding to the intact molecule, thus demonstrating the role of the 31-kD domain in affecting the binding to the 8-kD domain.

A manuscript describing the structural studies has been submitted for publication.

CEB-63: Our studies of this model leucine-zipper peptide show that its reversible monomer-dimer equilibrium is characterized by large negative changes in both enthalpy and entropy. The negative entropy change suggests hydrogen bonding or van der Waals forces as the most probable forces involved in the interaction. The enthalpy change also suggests that the dimer formation involves a significant coil-to-helix transition.

SIGNIFICANCE: The significance of all of the studies described above is the same: They present information significant in correlating physical properties of biological macromolecules to their structure and function. The work on HIV reverse transcriptase is particularly important because it presents a new quantitative method for studying various aspects of this enzyme, which has major biomedical significance.

PROPOSED COURSE: It is expected that work on these molecules and others of similar interest will be continued.

PUBLICATIONS: Green S, Ginsburg A, Lewis MS, Hensley P. Roles of metal ions in the maintainance of the tertiary and quaternary structure of arginase from *Saccharomyces cervisiae*. J Biol Chem 1991;266:21474-81.

Becerra SP, Kumar A, Lewis MS, Widen SG, Abbotts J, Karawya EM, Hughes SH, Shiloach J, Wilson SH. Protein-protein interactions of HIV-1 reverse transcriptase: implications of central and C-terminal regions in subunit binding. Biochemistry 1991;30:11707-19.

Yoo S-H, Lewis MS. Effects of pH and Ca^{2+} on monomer-dimer and monomer-tetramer equilibria of chromagranin A. J Biol Chem 1992;267:11236-41.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 RR 10204-08 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Cell Handling Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen Leighton, Sc.D.	Mechanical Engineer	BEIP, NCRR
Thomas Clem, Sr., BSEE	Electrical Engineer	BEIP, NCRR

COOPERATING UNITS *(If any)*
DCBD, NCI (J. Berzofsky)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In work on monoclonal antibodies, as well as in other endeavors, it is desirable to isolate individual cells. We are developing an inexpensive device for placing one, and only one, cell in each well of a microtitre tray that contains culture medium. Thus, the progeny in each well will all be descendents of that single cell (i.e., monoclonal). Our device is an improvement over the existing technique of limiting serial dilution, because each well contains a single cell, while a tray filled by the limiting serial dilution technique will contain many empty wells, and may also contain a few wells that contain two or three cells. The device will be fully automated, requiring minimal operator intervention.

OBJECTIVE: To provide a simple, inexpensive laboratory instrument capable of routinely injecting a single cell from a dilute suspension into each well of a microtitre tray. The instrument must function inside a sterile hood. In addition, the device must handle standard microtitre trays and be both easy to use and easy to keep clean.

METHODS EMPLOYED: A microprocessor-based, self-contained instrument has been designed and built. A unique, serpentine rack-and-pinion mechanism is used as the heart of a system for indexing a titre tray, placing each well in turn under a distribution nozzle, as in a fraction collector. The suspension of cells is diluted in such a way that the average drop at the nozzle tip will contain a single cell. However, rather than relying on Poisson statistics alone, the cell content of each drop is monitored by a microcomputer, which tracks the resistance changes as cells pass through a small orifice (similar to a Coulter counter). Drops that contain only one cell are allowed to fall into a well, and the tray is indexed to the next well. Drops that contain either no cells or more than one cell are removed, and the device waits for a single-cell drop before proceeding.

MAJOR FINDINGS: Thorough tests on the prototype instrument indicated that the Coulter orifice approach is insufficiently robust for the intent of this instrument. A variety of noise sources that are insignificant when counting thousands of cells become overwhelming when an absolute reliability at the single-cell level is required.

SIGNIFICANCE: The present approach must be abandoned, and an alternative method found.

PROPOSED COURSE: An alternate approach will be tested.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10214-08 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Photoradiation Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Paul D. Smith, Ph.D. Physicist BEIP, NCRR

COOPERATING UNITS *(if any)*
ROB, DCT, NCI (A. Russo, T. DeLaney); SB, DCT, NCI (H. Pass, W. Sindelar, M. Walther)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Photodynamic therapy (PDT), which is the interaction of light with hematoporphyrin derivative (HPD or Photofrin II), is in clinical use in several areas of application. Currently, PDT is being used clinically for bronchial obstructions, carcinoma *in situ* of the bladder, and intraperitoneal and pleural tumors. Specially designed and constructed fiber-optic probes and introducers are modified on a case-by-case basis and calibrated in terms of irradiance delivered to the tissue. Calibrated custom photodiodes are placed at strategic locations within the cavity to provide a dosimetry record and irradiance values (see project Z01 RR 10272-05). Dosimetry for PDT is complex, due to the variable irradiation levels within the tissue, the sequestering of HPD, and tissue oxygenation.

OBJECTIVES: To evaluate PDT as a modality for treatment of selected diseases. To enhance the effectiveness of PDT as a clinical procedure by reducing the complications associated with its use. To characterize tissue, permitting theoretical analyses that predict the irradiation level at any point in the tissue distant from the point of light injection.

METHODS EMPLOYED: Two argon-ion-laser pumped liquid-dye lasers produce up to 11W at 631nm. 15W can be delivered using 514-nm lines selected from the argon-ion output. All laser light is transmitted to the operating room by 600-u fused silica fibers, to which sterile fiber-optic probes are attached. Custom and commercial fiber-optic probes were developed from 200-u and 600-u fused silica fibers.

For bronchial obstructions and bladder work, specially designed fiber-optic probes were constructed to produce a uniform distribution in a cylindrical or spherical profile surrounding the fiber. Bronchial obstructions were treated through a bronchoscope in a fully anesthetized patient, with irradiance levels in the tumor of 400mW/cm, for a total delivered treatment of 250 J/cm. Tissue surrounding the tumor site was treated with 250mW/cm^2 to a total of 100J/cm^2. Follow-up bronchoscopy was performed 72 hours later to remove necrotic tissue.

Intraperitoneal and pleural therapy involve a wide range of tumor types. Light delivery is designed to treat all surfaces within these cavities, and similar techniques are used in both modalities. An initial surgical resection of tumor to a thickness not exceeding 5mm is performed, and all adhesions resulting from previous surgery are removed. Two methods of delivery are used: For the abdominal and pleural wall and organs, a wand consisting of an inflated tracheotomy tube filled with 0.5% intralipid solution, into which the treatment fiber(s) are inserted; and a surface illumination mode for the small bowel and mesentery. The wand removes all directionality of the laser light, and prevents an excessively high local irradiance of tissue. Power levels at the fiber tip are 2kW/cm^2, which can cause coagulation of the tissue and consequential damage of the fiber. If tissue breakdown product is present on the end of the fiber, the optical energy is absorbed at the tip with generation of heat. Photodiodes are sewn into various locations in the peritoneum and pleural cavity in order to monitor the light distribution. 0.2% intralipid is introduced into the cavity to help produce uniform illumination of the surfaces. 550-nm filters are used to cover the surgical lights to reduce the PDT effect from these lights during the extensive surgery required. Treatment levels have been increased intraperitoneally to an overall irradiance of 7.5J/cm^2 using 514-nm light, followed by irradiance of selected sites of tumor involvement using 630nm light up to a level of 157J/cm^2. At power levels delivered remotely from the laser by 600-μ fiber, treatment times typically last three hours.

Intrapleural therapy is performed exclusively using 630-nm light with irradiance levels increased to $35J/cm^2$. Typical laser treatment times are two hours.

In both pleural and intraperitoneal therapies, the treatment times are dictated by photodiode sensors sewn into the cavity to indicate when the desired level has been achieved. A PC-based computer provides both an on-line display of the irradiance levels achieved at seven strategic locations and a chronological, permanent record of the procedure on disk.

In the bladder, a spherically emitting fiber is used, which is positioned within the bladder (inflated to approximately 70% of full volume) with the aid of ultrasound imaging. Treatment times are typically 30 minutes long, producing a total dose of 3000 to 3500 J at 630nm. A prototype instrument is used to position 400-μ spherical sensors against the bladder wall. These sensors are being evaluated as an on-line dosimetry technique.

MAJOR FINDINGS: The clinical response to the bronchial treatment is encouraging for both total occlusion and narrowing of the bronchus. In all cases, the follow-up bronchoscopy removed necrotic tumor tissue.

A variety of tumor types have been treated in the intraperitoneal therapy with escalation of delivered dose. Response to the therapy is assessed by clinical symptoms and by analysis of cytological washings. Phase I dose escalation studies in both modalities are near completion, with the final irradiance levels indicated above.

Treatment times, even with two high-powered lasers, can reach four hours; tissue penetrance is limited to a maximum of 5mm with 630-nm light, and a maximum of 1mm with 514-nm light. The latter, even with higher power levels available and increased photodynamic effect due to more efficient absorption, can still lead to long treatment times if substantial blood contamination is present in the intralipid medium.

The photodiode monitoring system has been shown to be equivalent to irradiance levels based on the power-time product, and is now used exclusively to control therapy. Prime advantages of this system are its ability to account for fluctuations in laser power and for reduced irradiance due to blood contamination in the intralipid medium. A modified version is being evaluated for bladder work.

SIGNIFICANCE: Photoradiation therapy has been shown to be effective in certain instances, to have potential in others, and in some cases (e.g., pulmonary metastatic disease) to provide a unique approach to the treatment of cancer. Future acceptance of PDT will depend on several factors, some of which are addressed here. A procedure for assessing the appropriate level of

irradiation is essential in designing zones of treatment. In addition, identifying responsive diseases and defining treatment modalities for them is of prime importance with PDT.

PROPOSED COURSE: We will continue human clinical trials, and investigate animal models as necessary to explore new approaches. Light penetrance studies will be incorporated with singlet oxygen detection methods to define the levels necessary to cause the cytotoxic effects of PDT. Evaluation and interpretation of photodiode and other techniques for assessing light delivery will continue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10225-08 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Processing of High-Resolution Electron Micrographs

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Akram Aldroubi, Ph.D.	Staff Fellow	BEIP, NCRR
Michael Unser, Ph.D.	Visiting Scientist	BEIP, NCRR

COOPERATING UNITS *(if any)*
CSL, DCRT (B. Trus); LPB, NIAMS (A. Steven)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the years, we have developed several computational techniques for the statistical analysis of sets of electron micrographs of biological macromolecules. These methods include various types of factorial analysis (correspondence analysis, principal components), an outlier detection scheme, and clustering algorithms, as well as a statistical criterion for quantitative assessment of spatial resolution (spectral signal-to-noise ratio).

An aspect that has been considered in more detail is the problem of correcting for magnification mismatches between micrographs due to varying imaging conditions. The motivation is to be able to obtain higher-resolution 3-D reconstructions of icosahedral viruses by combining a larger number of micrographs with slight disparities in magnification. For this purpose, we derived a fast iterative algorithm that determines the scaling factors by matching auxiliary one-dimensional functions (radial autocorrelation) computed for each micrograph. We have also developed a spline-based algorithm for scale conversion with an arbitrary scaling factor. The procedure is such that it minimizes the loss of information.

OBJECTIVES: There are currently four main objectives in this research: (1) The determination of appropriate statistical models (noise and various distortions) for high-resolution micrographs; (2) the study of algorithms for the registration of specimen views using simple (translation/rotation) or more complex geometrical transformations; (3) the decision procedure that determines the classification of different views, as well as the detection of "bad" samples; and (4) the assessment of the quality of results obtained by averaging (e.g., signal-to-noise improvement, resolution). In particular, we have considered the problem of correcting for magnification mismatches between micrographs. Each image displays a series of independent projective views of the object under consideration. These views have different orientations and are not registered. Moreover, the micrographs may have different contrast.

METHODS EMPLOYED: The stretch factor between two micrographs is determined by comparing one-dimensional radial autocorrelation functions computed from each micrograph. These autocorrelation functions are matched using a fast iterative algorithm. The correction procedure is based on the principle of least squares spline approximation.

MAJOR FINDINGS: We have tested the method and used it for performing 3-D reconstructions. We have found that the algorithm is very fast, robust, and highly insensitive to noise. We have compared a 3-D reconstruction that used the factors computed by the algorithm, and one in which the factors were determined heuristically by an expert in the field, and found that our method is more reliable.

SIGNIFICANCE: The acquired scientific expertise and the developed software should allow other scientists to use these image processing and computer reconstruction techniques to determine or understand a specimen's structure. The derived methods and the developed software constitute an important step in obtaining higher-resolution 3-D reconstruction of macromolecules.

PROPOSED COURSE: We have planned to develop statistical procedures for assessing symmetry properties and for the objective comparison of putative subsets of particles. Another problem is to correct for the effect of the contrast transfer function when combining several micrographs.

PUBLICATIONS: Steven AC, Kocsis E, Unser M, Trus BL. Spatial disorders and computational cures. Int J Biol Macromol 1991;13:174-80.

Trus BL, Unser M, Pun T, Steven AC. Digital image processing of electron micrographs: the PIC system II. Scanning microscopy (in press).

Aldroubi A, Trus BL, Unser M, Booy FP, Steven AC. Magnification mismatches between micrographs: corrective procedures and implications for structural analysis. Ultramicroscopy (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10256-06 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanical Prosthetic Heart Valve Tester

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas L. Talbot, M.S. Mechanical Engineer BEIP, NCRR

COOPERATING UNITS *(if any)*
FDA (S. Stewart); NHLBI

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Applied Clinical Engineering Section

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TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Six different manufacturers' prosthetic heart valves were tested and ranked according to performance indices described by Swanson and Gabbay. Additionally, a technique was devised whereby the pressure drop across the aortic valve could be predicted by velocity measurements made with continuous-wave Doppler (CWD) imaging. The results of preliminary *in vitro* studies demonstrate a high correlation ($r>0.9$) between the peak velocity as measured with CWD and the predicted velocity obtained by the application of a simplified Bernoulli equation, which uses a measured maximum pressure drop across the aortic valve. Also, an acoustical signature analysis technique is under investigation for possible noninvasive detection of partially failed single-leaflet-type mechanical valves.

PROPOSED COURSE: The system works well with mechanical prosthetic valves, and the data acquisition and analysis portions of the project have been refined considerably. Plans include the modification of the system to facilitate the testing of explanted mechanical and bioprosthetic heart valves. Additional analysis has revealed errors in measuring prosthetic valve pressure gradients by continuous-wave Doppler, compared to standard catheter measurements. These errors appear systematic, and vary depending on the type of prosthetic valve. One other manuscript detailing these observations and their clinical implications is under preparation.

PUBLICATIONS:

Stewart SFC, Nast EP, Arabia FA, Talbot TL, Proschan M, Clark RE. Errors in pressure gradient measurement by continuous-wave Doppler: type, size, and age effects in bioprosthetic aortic valves. J Am Coll Cardiol 1991;18:769-80.

Nast EP, Stewart SFC, Arabia FA, Talbot TL, Clark RE. Doppler-derived gradients across prosthetic aortic valves are inaccurate. Surgical Forum 1990;41:223-5.

Arabia FA, Talbot TL, Stewart SFC, Nast EP, Clark RE. A computerized physiologic pulse duplicator for *in vitro* hydrodynamic and ultrasonic studies of prosthetic valves. Biomed Instrum Technol 1989;23:205-15.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10257-06 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Analysis of Propagation of Light in Turbid Biological Tissues

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Robert F. Bonner, Ph.D.	Physicist	BEIP, NCRR
Joseph M. Schmitt, Ph.D.	Staff Fellow	BEIP, NCRR

COOPERATING UNITS (if any)
LPS, DCRT (R. Nossal, G. Weiss, A. Gandjbakhche); Department of Physics, Bar-Ilan University, Israel

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INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

Many of the clinical research projects of the laser and modern optics group involve the interaction of light with tissue (e.g., laser microsurgery, laser Doppler blood-flow measurements, photodynamic therapy of cancer, noninvasive platelet assessment, and optical tissue oximetry). In order to quantitate these techniques more fully, we have undertaken theoretical modeling of light propagation in biological tissues and turbid media.

Analytical equations have been devised characterizing various parameters of photons illuminating a tissue surface (including probability of surface re-emission at a given distance, mean path before re-emission, mean depth of penetration, and probability of absorption with depth). These expressions have been used to interpret empirical measurements on living tissues, and to quantify a variety of clinical measurements (e.g., laser Doppler blood-flow and volume measurements, dosimetry in PDT of cancer, and remote sensing of atherosclerotic plaque).

Recently our theoretical predictions of path length distributions of re-emitted photons have been applied to temporal dispersion of picosecond laser pulses in brain and muscle. Such analysis may allow noninvasive, absolute quantitation of hemoglobin and myoglobin oxygen saturation *in vivo*, as well as the concentration of a variety of important biomolecules.

OBJECTIVES: To develop insight into light propagation in turbid homogeneous and layered biological tissues, and to provide analytical expressions of general utility.

METHOD EMPLOYED: Mathematical theory of random walks on lattices and both Monte Carlo and exact enumeration computer modeling have been used to develop a quantitative analysis of light propagation in biological tissues. Empirical measurements have been obtained from tissues using laser fiber-optic systems. Comparison with diffusion theory has been examined.

MAJOR FINDING: A set of analytical equations has been developed that is generally useful when illuminating a tissue surface. New scaling relationships have been quantified for light propagation with different anisotrophy of scattering (i.e., similarity relations in analytical expressions for $\langle \cos \theta \rangle$, u_s, and u_a).

SIGNIFICANCE: Light is frequently used to probe and treat biological tissues; however, accurate evaluation of light propagation in tissue has yielded few clinically useful insights or relationships. As a result, most interpretation either has been qualitative or has required extensive correlations with techniques that have poorly controlled optical variations. Our analytical theory gives readily applied equations for a variety of optical parameters of photon propagation in tissue.

PUBLICATIONS:

Bonner RF, Nossal R, Weiss GH. A random-walk theory of time-resolved optical absorption spectroscopy in tissue. In: Chance B, ed. Photon migration in tissue. New York: Plenum, 1991;11-23.

Gandjbakhche AH, Bonner RF, Nossal R. Scaling relationships for anisotropic random walks. J Stat Phys 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10258-06 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Photochemical Inactivation of Virus and Bacteria in Blood

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Robert F. Bonner, Ph.D. Physicist BEIP, NCRR

COOPERATING UNITS *(if any)*
DBBP, FDA (K. Prodouz, J. Fratantoni); ROB, NCI

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

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INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Photochemical sterilization of blood products is the goal of this project, in which we are quantitating photochemical destruction of virus in blood and blood products, and the effects of the process on the native blood components. Initial studies using an XeCl excimer laser characterized the response of virus and blood components as functions of cumulative fluence and peak irradiance. Single- and multiple-photon photochemical schemes were investigated. Single-photon photochemistry resulted in an efficacious treatment range between 10-20 J/cm^2, in which a hardy virus is inactivated and platelets and plasma proteins are minimally affected. Multiple-photon effects at higher irradiance led to increased protein damage in blood components, without increasing efficacy of viral inactivation. Augmentation of this therapeutic window by photochemistry (using exogenous agents such as riboflavin, tetracycline, and hemocyanin) was investigated, with a focus on the adverse effects on platelets and the relative susceptibility of different viruses dependent on the complexity of their coats. The inhibitory effect of serum albumin on photochemistry was also examined. Proposed work will examine exogenous agents that show strong affinity for binding to DNA and thereby might increase therapeutic efficacy. The high sensitivity of blood platelets to activation and/or damage by photochemical products (e.g., singlet oxygen) has led to a renewed emphasis on optical and immunological methods of monitoring minimal lesions associated with viral inactivation photochemistry, as well as standard blood-banking techniques of preparation and storage.

OBJECTIVES: To develop an efficacious means to inactivate viral and bacterial agents in blood.

METHOD EMPLOYED: Photochemical treatment, using either UV-laser effects on DNA or visible light sources interacting with exogenous photochemicals. Quantitative dosimetry and assays of viral inactivation and blood component function were used to determine dose response curves. Noninvasive optical assays, using low-angle, multiple scattering of flow-oriented platelet suspensions, allowed *in vitro* determination of functional changes in platelets.

MAJOR FINDINGS: UV-laser photochemical inactivation of virus has a therapeutic window of efficacy (10-20 J/cm^2 @ 308nm and ≤ 0.2 MW/cm^2) in which platelet and plasma protein function are maintained. Two-photon photochemistry at higher irradiance (~1.4 MW/cm^2) results in little improvement in viral inactivation, but dramatic loss in blood component function, presumably due to peptide bond breakage. Sensitivity of blood cells (e.g., platelets) and the strong binding and/or quenching by serum albumin have proven to be severe limitations of the use of exogenous agents exhibiting non-DNA-specific photochemistry. Viral inactivation is also very sensitive to viral coat composition and its permeability to the exogenous agents.

SIGNIFICANCE: Transfusion risks associated with the transmission of infectious agents remain a major problem in transfusion medicine. A simple, effective, noninvasive method to inactivate these infectious agents would be of enormous clinical importance. Possible patient treatment procedures can be envisioned, using photochemical treatment of the blood of a patient during plasmapheresis.

PROPOSED COURSE: Experiments with psoralen photochemistry *in vivo* will explore complex intravascular bioeffects. A new emphasis will be optimized use of the blood supply, minimizing the number of donors to which a typical patient is exposed, thereby reducing cumulative risks. A comparative study, in which new immunological methods to sense subtle changes in platelet membrane function will be correlated with noninvasive optical assessment, seeks to elucidate and refine rapid, routine noninvasive optical analysis of blood products.

PUBLICATIONS: Prodouz KN, Lytle D, Bonner RF, Fratantoni JC. Effects of two viral inactivation methods on platelets: laser-UV radiation and merocyanine 540-mediated photoinactivation. Blood Cells 1992;18:101-16.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10259-06 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Visual Target Tracking Ability Assessment System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas R. Clem, Sr., BSEE Electronic Engineer EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LPP, CNSB, NIMH (R. Litman); LSES, NIMH (C. Schooler, B. Roberts)

LAB/BRANCH
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INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.9	0.1

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects _(b) Human tissues (c) Neither

_(a1) Minors
_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Abnormal eye movements found in patients with central nervous system (CNS) diseases can sometimes be revealing about the underlying pathology. Although the oculomotor nuclei, their descending primary motor neurons, and the extraocular muscles they innervate ultimately mediate eye movements, higher centers (including premotor areas in the brain stem, midbrain, subcortical areas, and cerebral cortex) are also involved in the movements. The oculomotor nuclei and neurons, together with the higher motor centers, functionally comprise five oculomotor systems responsible for different types of eye movements. Of these, the smooth pursuit eye movement system and saccadic eye movement system are of interest, for they seem to be abnormal in CNS disorders where disease in higher subcortical and cortical centers is implicated.

Moreover, both smooth pursuit and saccadic eye movements have distinct and easily measured properties. Disorders of smooth pursuit eye movements (SPEM) have been found in schizophrenia, CNS illnesses affecting the oculomotor nuclei (e.g., brainstem infarction), diffuse brain diseases (such as disorders of the Alzheimer's type), Huntington's disease, and Parkinson's disease.

OBJECTIVE: To study the ability of subjects to follow a variety of target movements, to compare the results of normal subjects with those of subjects known to have dementia, and to define a set of parameters that will characterize abnormal conditions. A portable system has been fabricated that allows researchers to study patients and their family members in their homes or at a medical facility remote to the NIH. Tests are also being given to AIDS patients to assess the effects of the HIV on the CNS.

METHODS EMPLOYED: A custom interface was built to interconnect a commercial eye position detector and a desktop PC. A second monitor connected to the PC provides the moving target, a small spot moved horizontally in a variety of patterns. Custom programs were written to produce the desired moving target pattern and to collect the eye position data. These programs allow graphic display of the data and some rudimentary analysis.

SIGNIFICANCE: This system allows researchers to acquire data that were unobtainable prior to its development. The use of the PC provides the advantages of flexibility of experimental parameters, speed and accuracy of data acquisition, simultaneous acquisition of data from several sources, and permanent storage of raw data.

PROPOSED COURSE: As further data are acquired, new target challenges will be tried. We intend to continue this program of refinement, and to alter our procedures as the results of our experiments direct. The system is being upgraded with a newer, faster computer. Improvements of data analysis and data reporting are being developed. Further tests and equipment are being developed to test a subject's abilities to coordinate eye movements with hand responses and auditory stimuli. Programs will be enhanced to allow multiple target presentations, including moving distractor images, while maintaining the 1-millisecond sample rate of eye position data acquisition; and to produce multiple color displays, using techniques to modify individual pixel attributes on the display. The electronic interface electronics will be modified to allow the use of the system with VGA video systems.

PUBLICATIONS: Hommer D, Clem T, Litman R, Pickar D. Maladaptive anticipatory saccades in schizophrenia and the utilization of representational knowledge. Biol Psychiatry 1991;30:779-94.

Litman RE, Hommer DW, Clem TR, Ornsteen ML, Ollo C, Pickar D. Wisconsi card sort performance correlates with eye tracking in schizophrenia. Am J of Psychiatry 1991;148:1580-2.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10260-06 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Real-Time High-Performance Confocal Laser Scanning Optical Microscope

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Seth Goldstein, Sc.D. Chief, MES BEIP, NCRR

COOPERATING UNITS *(if any)*
LNP, NINDS (T. Smith)

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INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A no-moving-parts, 30-frames-per-second, laser beam scanning confocal reflected light and fluorescence microscope has been developed. In principle, the technique could be extended to transmission light microscopy. Acousto-optic beam deflectors, controlled by all-digital electronics, move a laser beam in a 512-line, interlaced raster. The light enters an inverted microscope through the epifluorescence side camera port, and is imaged at the object by the microscope objective. Reflected or fluorescent light returns through the objective, exits through the camera port, and is imaged onto the photocathode of an image dissector tube (IDT). Confocality is provided by raster-scanning the IDT aperture coincident with the congruent image of the laser beam incident on the object. Real-time, jitter-free reflected and fluorescent light images of a variety of biological objects have been produced.

OBJECTIVES: To develop a greatly improved confocal laser microscope with no moving parts, that will retain the optical performance of existing confocal microscopes, yet generate images at video rates and be self-aligning under computer control.

SIGNIFICANCE: The superior optical performance of the confocal microscope is now widely recognized in the biomedical community. Most commercially available systems use galvanometer laser scanners to generate the picture, and are therefore slow--typically yielding images at an order of magnitude less than video rates. Commercial systems operating at video rates are only confocal in one, rather than two, dimensions (with attendant disadvantages). Our system operates at TV-frame rates (partial fields operate at faster speed), and is confocal in both dimensions. The rapid operating speed should enhance ease of use and allow studies of rapidly occurring biological phenomena. The lack of moving parts is anticipated to result in greatly reduced maintenance and alignment problems, compared to existing units.

METHODS EMPLOYED: A very precise and repeatable raster scan of a laser spot is performed with two acousto-optic beam deflectors under digital control. A novel means of scanning a pinhole effectively in synchrony and alignment with the laser beam, using an image dissector tube, has been developed and patented. Light getting through the pinhole is sensed, and used to modulate a raster scan on a TV monitor to generate the confocal image. The increase in speed is achieved by scanning the laser beam, rather than mechanically scanning the object through a stationary laser beam. A personal computer controls all alignment procedures, as well as image generation. The system has no moving mechanical parts except for the axial focus. A new laser scanner, utilizing anamorphic prism beam expanders and contractors, has recently been completed and is predicted to yield diffraction-limited performance.

PROPOSED COURSE: The improved laser scanning system was built, and a higher-powered laser was installed to compensate for system light losses. Uses for reflected light and fluorescence are being evaluated. The project is essentially complete, except for minor modifications.

PUBLICATION: Goldstein S, Hubin T, Smith T. An improved no-moving-parts video-rate confocal microscope. In: Acharya RS, Cogswell CJ, Goldgof DB, eds. Biomedical image processing and three-dimensional microscopy (proceedings of the Society of Photo-Optical Instrumentation Engineers). Bellingham, Washington: The International Society for Optical Engineering, 1992;1660:456-65.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10272-05 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Photometry for Photodynamic Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR
John Cole, MSEE	Electronic Engineer	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
Robert F. Bonner, Ph.D.	Biophysicist	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
ROB, DCT, NCI (A. Russo, T. Delaney); SB, DCT, NCI (H. Pass)

LAB/BRANCH
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SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)
X _(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Dosimetry for photodynamic therapy (PDT) involves measurement of hematoporphyrin derivative (HPD) concentration, oxygen concentration, singlet oxygen concentration, and therapeutic light level, all as a function of position throughout the tissue being treated either intentionally or unavoidably. Noninvasive measurements are essentially limited to surfaces, although theoretical and empirical models can be used to estimate values some distance into the tissue. Fiber-optic probes can be used for direct subsurface measurements.

OBJECTIVE: To quantitate principal parameters involved in PDT in experimental models, animal trials, and human treatment.

METHODS EMPLOYED: A small handheld probe was developed to measure surface concentration of HPD. The excitation is blue light from a coherent model PRT-100 laser, electromechanically chopped at 5hz and conveyed to the tip of the probe by an optical fiber. The tip is conical, with an aperture 2mm in diameter, allowing good spatial localization. Fluorescence of the HPD is separated from reflected excitation and tissue fluorescence by two filters, and sensed by a solid-state photodiode. The component at the chopped frequency is amplified and read by a digital voltmeter or, for very low-level measurements, by a lock-in amplifier. During the past year additional experimental usage and verification have been carried out, and use for other types of measurements has been explored.

For measurement of light delivered during intraperitoneal treatments, a twelve-channel, computer-based light monitoring system has been designed and built. Completely insulated solid-state photodiodes connected by coaxial cables are sutured into positions of interest within the abdominal cavity. The computer applies calibration constants for each diode, and integrates the light dose at each site in real time. Multiple isolation transformers and special construction features insure freedom from electric shock hazard.

Several new designs of photodiode assemblies have been developed and are being evaluated. A different type of interface box has been designed and built to allow integrating spheres at the ends of optical fibers to be used as the sensors. This is basically a different type of measurement, and work is under way to correlate the readings obtained by the two types of sensors. The spherical sensors are essential for monitoring light delivery for bladder cases, which are also monitored in real time.

MAJOR FINDINGS: The HPD probe is useful for assessing the distribution of HPD and its selective concentration in malignant tissue. The light monitoring system has been particularly useful for evaluating intralipid as a light-diffusing agent, and for quantitating the attenuation caused by the intralipid and blood. Useful information is also obtained on the light levels resulting from the operating room lights and surgeon's head lights. In addition to patient use, this system has been used in several laboratory studies to provide quantitative data.

SIGNIFICANCE: Data taken during dozens of human procedures have resulted in a substantial revision in the apportioning of light between different organs. In most instances, the real-time integrated values are now being used to control the duration of treatment. The results also show clearly the effect of leakage of blood into the intralipid, thus providing a sound basis for deciding when to change intralipid.

PROPOSED COURSE: Further software developments will make the system more versatile and easier to use. Additional theoretical and experimental work is needed to provide a more accurate relationship between measured values and the actual effective dose.

PUBLICATIONS: DeLaney TF, Sindelar WF, Smith PD, Friauf WS, Pass HI, Russo A, Thomas G, Dachowski L, Cole J, Glatstein E. Initial experience with photodynamic therapy for intraperitoneal carcinomatosis. In: Sharp F, Mason W, Leake R, eds. Ovarian cancer: biological and therapeutic challenges. London: Chapman and Hall Medical, 1990;371-80.

Friauf WS, Smith PE, Russo A, DeLaney TF, Pass HI, Cole JW, Gibson CC, Sindelar WF, Thomas G. Light monitoring in photodynamic therapy. In: Nagle HT, Tomkins WJ, eds. 1991 IEEE case studies In medical instrument design. New York: IEEE, 1992;127-38.

Pass H, Tochner Z, Smith P, Friauf W, Glatstein E, Travis W. Intraoperative photodynamic therapy for malignant mesothelioma (letter). Ann Thorac Surg 1990;50:687-8.

Pass H, DeLaney T, Russo A, Mitchell J, Smith P, Friauf W, Thomas G. Feasibility of intrapleural photodynamic therapy: the first eight patients. In: Proceedings of optical methods for tumor treatment and detection: mechanisms and techniques in photodynamic therapy. Bellingham, WA: Society of Photo-Optical Instrumentation Engineers 1992;1645:2-9.

INVENTIONS: An invention report on a photodiode assembly has been filed.

PATENTS: A patent application for the handheld probe is in process.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10276-05 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microdialysis Probe Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Peter M. Bungay, Ph.D.	Chemical Engineer	BEIP, NCRR
Robert L. Dedrick, Ph.D.	Chemical Engineer	BEIP, NCRR
Paul F. Morrison, Ph.D.	Physical Scientist	BEIP, NCRR
Kevin H. Dykstra, Ph.D.	Chemical Engineer	BEIP, NCRR

COOPERATING UNITS *(if any)*
Experimental Therapeutics Branch, NIMH (J. Hsiao, I. Mefford)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

X _(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Microdialysis probe technology provides access to tissue interstitium for either sampling of diffusible tissue constituents or for delivery of bioactive substances; however, the relationship of the probe perfusate concentrations to their tissue counterparts is a complex function of solute molecular weight, solute physicochemical properties, tissue properties, probe membrane properties, probe geometry and perfusion rate, and the trauma of probe insertion into the tissue. These dependencies are being studied in order to improve the quantitative usefulness of the technology. The focus of applications is the brain in connection with diseases such as Parkinson's, AIDS, and Alzheimer's. Endogenous solutes of interest include dopamine and its metabolites. Exogenous substances employed as marker solutes or pharmacological agents are acetaminophen, AZT, and sucrose. Mathematical modeling is being used to describe solute transport within the probe and surrounding medium. Validation experiments involve tissue autoradiography and histology, as well as measurement of perfusate concentrations.

PHS 6040 (Rev. 5/92)

RELEVANT BEIP PROJECTS: 88-111, Microdialysis Probe Studies; 91-140, Intrathecal AZT for AIDS Dementia.

OBJECTIVES: The first set of general objectives is to understand and better describe the factors that affect microdialysis performance in order to improve the usefulness of the technology and expand its capabilities. The term *performance* is used here to refer to the relationship between microdialysis perfusate concentrations and those in the medium surrounding the probe, i.e., the tissue in *in vivo* situations. A second set of objectives concerns specific applications in neuroscience research and studies of diseases such as Parkinson's disease and AIDS dementia.

METHODS EMPLOYED: A microdialysis probe contains a synthetic membrane, typically in a cylindrical capillary configuration. The inside of the probe is perfused with a dialysate medium. The effluent perfusate is either passed by a detector or collected for subsequent assay of the constituents that have crossed the membrane to (or from) the medium bathing the external surface of the membrane.

Unless the perfusion medium (dialysate) is allowed to equilibrate with the external medium, the activity of the solutes in the perfusate will differ from the corresponding exterior activities. This is because of gradients in solute activity within the dialysate, membrane, and tissue inherent to the diffusional exchange of solute between the tissue and the dialysate. In general, empirical *in vitro* calibration of a probe cannot be relied upon in *in vivo* applications because of the difficulty of simulating tissue with respect to transport characteristics, binding, metabolism, vascularization, and trauma of probe insertion.

The use of mathematical modeling is being investigated for simulating solute transport within the blood, tissue, and probe compartments. The probes are characterized in *in vitro* studies. *In vivo* studies in the brains of anesthetized rats utilize model compounds to aid in understanding transport through tissue and the perturbation caused by the presence of the probe. Available assay technologies include HPLC, electrochemical and spectrophotometric detection, and scintillation counting. In addition, an autoradiography technique has been developed to measure the spatial variation in solute concentration produced by the probe.

MAJOR FINDINGS: General mathematical treatments for quantitative microdialysis have been proposed. For steady-state operation of a continuously perfused probe, expressions have been derived for the dialysate, membrane, and external medium mass transfer resistances. For *in vivo* applications, solute generation and catabolism in the tissue (if present) are assumed to follow zero-order and first-order kinetics, respectively; and extracellular-to-intracellular and extracellular-to-microvascular exchange are assumed to be linear. For *in vitro* applications, expressions

derived for quiescent and well-stirred external media demonstrate how strongly dialysate concentration can be affected by convection in the external medium.

The resistances were evaluated for the sampling of tritiated water from rat brains, and the results were used to compare theoretical predictions to quasi-steady-state data from the literature. Excellent agreement was obtained between theory and experimentation for the variation in dialysate effluent concentrations with dialysate flow rate. The theory clarified the origin of the discrepancy between *in vivo* and *in vitro* microdialysis measurements.

An annular flow apparatus for *in vitro* probe characterization was constructed, in which the degree of convection in the external medium can be varied in a controlled and describable manner. Studies have been performed with this apparatus, using acetaminophen as the marker solute.

A general solution describing transient probe-tissue behavior was also obtained. Experiments were performed in which acetaminophen was administered to rats by bolus intravenous injections. In one set of experiments, microdialysis probes were used to sample acetaminophen from the caudate; in another, anesthetized rats were sacrificed at various post-injection time points to obtain brain tissue samples for assay. Very good agreement was obtained between the predicted and observed time courses of both the dialysate effluent and total tissue drug concentrations.

Thus, specific models derived within the proposed general modeling framework have been tested for both transient and steady-state probe operation. Additional simulations were performed for a variety of small solutes of interest: cis-platin, dopamine, dihydroxyphenyl acetic acid (DOPAC), glucose, and adenosine.

The mathematical modeling efforts have been extended in several ways. One way was to develop simplified solutions in cylindrical geometry for the dialysate and membrane compartments, collapsed into a boundary condition for the external medium compartment. Whereas the original cylindrical coordinates transient solution was limited to low values of dialysate extraction fraction, the simplified solutions are valid over a wide range of extraction fractions. In addition, a corresponding transient solution in spherical coordinates was developed to assist in examining the importance of end effects. All of these model variations are restricted to one solute, and the extracellular and intracellular spaces have been coupled by the assumption of rapid exchange between these compartments. The various models are being incorporated into MICRODIAL, a Macintosh program, suitable for exporting to the microdialysis user community.

A series of *in vivo* validation experiments was conducted involving the administration of the extracellular marker [^{14}C]-sucrose

through microdialysis probes into rat caudate. Autoradiography was used to follow the time course of dialysate extraction fraction, and to determine the radially varying concentration profile as a test of predictions from the mathematical models. Both types of measurements were consistent in suggesting that an edematous region of 35% to 40% extracellular volume fraction surrounded the probes and extended beyond the approximate 3-mm-diameter interval over which the sucrose levels were detectable.

Microdialysis is currently being used to study transport within the brain of the AIDS drug, AZT. These studies were undertaken in support of a proposed clinical trial for intraventricular administration of AZT to patients suffering from AIDS-related dementia. Microdialysis probes are being used to deliver the drug to rat brain parenchyma. Autoradiographs of the tissue surrounding the probes are being examined for indications as to whether an intraventricularly administered drug could reach therapeutic levels throughout the brains of patients. These studies are also examining AZT metabolism in rat brain and the influence of systemically administered probenecid on AZT transport.

Experiments have begun that involve infusion of radiolabeled indole acetic acid (IAA) through probes implanted in rat caudate with and without the presence of probenecid. Indole acetic acid is a model compound for the acid metabolites of seratonin and dopamine. This study is intended, in part, as a demonstration of the effect of tissue clearance processes on microdialysis measurements. Probenecid, an agent which blocks active transport mechanisms, is expected to alter the active efflux of IAA from brain interstitium to blood.

In the course of preparing to perform microdialysis measurements on dopamine and its common metabolites, a potential new metabolite of dopamine was identified; its production from dihydroxyphenyl acetic acid (DOPAC) is catalyzed by copper and manganese ions. This new compound is of special interest, because poisoning by these two metals produces lesions similar to those observed in Parkinson's disease.

SIGNIFICANCE: Considerable interest is being shown in microdialysis because of its potential application to a variety of tissues and a wide range of solutes. The technology is not solute-specific, and perfusate samples are free of macromolecules; consequently, various separation and analysis techniques can be employed in the subsequent sample processing. However, the empirical manner in which the technology has been applied has limited its utility and hampered interpretation of measurements. The mathematical models developed in this project provide a rational basis for planning and interpreting microdialysis experiments in ways that would not be feasible otherwise.

PROPOSED COURSE: Work will continue on several of the studies mentioned above, notably *in vitro* probe characterization and

pharmacokinetics of both AZT and IAA. Efforts will be made to modify the general models. Desirable modifications include multicompartment extensions allowing for intermediate rates of exchange between the extracellular and intracellular spaces; treatment of coupled multiple species, e.g., a parent compound and its metabolites; and inclusion of nonlinear exchange and metabolic characteristics. Such generalizations would permit more realistic models for solutes of considerable interest--for example, neurotransmitters (dopamine in particular). Other desirable extensions include incorporating axial, as well as radial, diffusion to investigate the importance of end effects.

PUBLICATIONS: Morrison PF, Bungay PM, Hsiao JK, Mefford IN, Dykstra K, Dedrick RL. Quantitative microdialysis. In: Justice J, Robinson T, eds. Microdialysis in the neurosciences, volume 7: techniques in the behavioral and neural sciences. Amsterdam: Elsevier Science Publishers B.V., 1991;47-80.

Dykstra KH, Hsiao JK, Morrison PF, Bungay PM, Mefford IN, Scully MM, Dedrick RL. Quantitative examination of tissue concentration profiles associated with microdialysis. Journal of Neurochemistry 1992;58:931-40.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10285-05 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Calorimetric Investigation of DNA/Anthracycline Drug Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Courtney P. Mudd, Ph.D. Biomedical Engineer ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*
Dept. of Chem., Rutgers Univ. (K. Breslauer); FDA (D. Remeta)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

After performing the first direct measurements of the binding enthalpies of the anthracycline drug daunomycin to DNA in the 10 to 20 micromolar range (where the monomeric form predominates), we extended the experiment to study the interaction with other DNA hosts at varying salt concentrations and temperatures. With a differential stopped-flow microcalorimeter, we were able to measure 120 of these enthalpies per day, with an uncertainty of only 3 microjoules. The enthalpy heats ranged from -20 microjoules (endothermic) to +50 microjoules (exothermic).

OBJECTIVE: To measure the binding heats to daunomycin to a variety of DNA hosts with drug concentrations in the 10 to 20 micromolar range (the range of therapeutic dosage). The DNA hosts we used were poly d(A)*d(T), poly d(G)*(C), salmon testes DNA, and calf thymus DNA. In addition, we also varied the salt concentration from 20 mM to 500 mM to investigate the salt dependence of the interaction, and varied the temperature from 0°C to 50°C to investigate the temperature sensitivity of these reactions. The binding enthalpies are good indicators of the drug's effectiveness in chemotherapy.

SIGNIFICANCE: With the sensitivity of our tantalum stopped-flow calorimeter, we can use sufficiently low drug concentrations to ensure that the monomeric form of the drug predominates. These experiments represented the first direct measurement of these heats at therapeutic levels. Previous measurements were performed at higher concentrations (for higher heat output), and corrections were used to account for drug aggregation at higher concentrations. Measurement of daunomycin's effectiveness at therapeutic levels is extremely important, due to the drug's toxic side effects. At present, daunomycin is the second most often used drug in chemotherapy.

RESULTS: Preliminary results indicate that the degree of salt dependence varies according to the type of DNA host used. Also, at these monomeric levels, the results show that the corrections used in earlier works (at higher concentrations) contained considerable errors in some cases. We are in the process of reducing the data for the binding profiles and their temperature sensitivities.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10286-05 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
High-Speed Differential Stopped-Flow Calorimeter

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Courtney P. Mudd, Ph.D.	Biomedical Engineer	ACES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electronics Engineer	EEES, BEIP, NCRR
Stephen B. Leighton, Sc.D.	Mechanical Engineer	MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
IR BC, NHLBI (R. Berger)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A dual-channel, differential, high-speed, stopped-flow calorimeter has been constructed to study the effects of mixing speeds, flow velocities, and pressure drops on the thermal and optical time course of chemical reactions. The system uses a variable-speed flywheel and an electrically actuated clutch to transfer sufficient energy to the four drive syringes to mix reagent volumes of 200 microliters in 5 milliseconds or less. The inlet tubes from the drive syringes, as well as the mixer and the detection chamber, are kept at constant temperature between 25° and 50°C. An ultrafast thermistor (1 ms) is located in the detection chamber to measure the temperature rise during the reaction. The drive cam profile produces a constant acceleration of the syringes. An optically transparent mixing chamber allows optical measurement of the reaction. The observation tube is now made of cast urethane that has been coated with a 1000-Å-thick layer of tantalum to prevent water vapor migration. The observation tube "floats" in O-ring seals to reduce shock loads during mixing. A linear optical encoder is used to measure the displacement of the drive syringes.

OBJECTIVE: The original objectives of the single-channel instrument remain the same: (1) to mix two 200-microliter reagent samples in less than 5 milliseconds; (2) to measure the heat of reaction after mixing; (3) to measure the pressure drop across the mixer in order to evaluate mixing efficiency as a function of flow velocity; (4) to vary the mixing time from fewer than 10 ms to more than 100 ms; (5) to allow optical measurement of the reaction; and (6) to control the temperature of the reaction over the range of 25° to 50°C. After evaluation of the thermal sensitivity of the single channel, we found the predominate source of uncertainty to be temperature variations in the incoming reagents. The addition of a second channel connected in opposition (differential operation) to the first channel causes this incoming temperature variation to become a common-mode signal. The differential scheme significantly reduced this uncertainty and produced an enhanced differential sensitivity.

SIGNIFICANCE: The high-speed mixing capability of the instrument allows the direct measurement of reaction kinetics in the millisecond range, with both thermal and optical detection schemes.

PROPOSED COURSE: We plan to use a microcomputer to acquire, synchronize, and store the data from the transducers used in this instrument.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10296-05 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Experiments with a High-Resolution Field-Emission STEM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	BEIP, NCRR

COOPERATING UNITS *(If any)*
LN, NINDS (S. B. Andrews); LSBR, NIAMS (F. Booy); Lehigh Univ. (J. Hunt); Baylor Col. of Med. (W. Chiu)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Further experiments have been performed to assess new applications of parallel-detection electron energy loss spectroscopy in the field-emission STEM. This technique provides a high sensitivity for microanalysis of certain important biological elements (such as calcium), the physiological concentrations of which are rather low in cells. Application of parallel-EELS mapping to the analysis of freeze-dried cryosections provides a means of detecting small amounts of calcium in structures with a diameter of ~50 nm. Detector pattern noise due to channel gain variations can be reduced by acquiring difference spectra at each pixel. By segmenting nitrogen maps that reflect the structure through the protein distribution, it is possible to sum spectra from specific compartments. It has been found that useful data can be collected at 100 keV beam energy from freeze-dried cryosections cut to a nominal thickness of 100 nm. The analysis results in a sensitivity of ±0.4 mmol Ca/kg dry weight, with a total acquisition time of 400 seconds, a significant improvement over that achievable with energy-dispersive x-ray spectroscopy. Another application of EELS is the measurement of precise protein crystal thickness for application to electron crystallography. This approach has been tested on glucose-embedded crotoxin, and results show that the thickness determination can be performed to an accuracy of one-half a unit cell.

OBJECTIVES: To develop parallel-EELS mapping techniques for obtaining the distributions of calcium in small subcellular compartments of rapidly frozen, cryosectioned tissue; and to investigate other high-resolution applications of EELS in the field-emission STEM.

METHODS EMPLOYED: The EELS data were obtained with a specially adapted magnetic sector spectrometer, equipped with a photodiode array parallel detector. Frozen-hydrated cyosections of mouse cerebellar cortex supported on grids were cryotransferred into the VG Microscopes STEM and were freeze-dried. Spectrum-images were recorded with up to 128 x 128 pixels, each containing 1024 energy channels by means of a PC486-based digital acquisition system. Processing was performed with both the PC486 system and a Macintosh II computer.

MAJOR FINDINGS: By applying difference-acquisition techniques, filtering, and segmentation techniques, it is possible to measure calcium at the few-parts-per-million level in structures with complicated shapes, such as the cisternae of endoplasmic reticulum. When the pixel size is only ~10 nm, this measurement translates into detection of only a few hundred atoms of calcium in the analyzed volume. Moreover, spectrum imaging allows analyses to be performed in an unbiased way from extended areas of the specimen.

The low-dose thickness measurement of glucose-embedded protein crystals using the EELS low-loss intensity shows that the determination can be made very precisely, within one-half of a unit cell.

SIGNIFICANCE: The parallel-EELS spectrum imaging technique can be used to detect physiological concentrations of calcium in cryosections of directly frozen tissue at a beam energy of 100 keV in the dedicated field-emission STEM. Experimental data suggest that the sensitivity for calcium analysis by EELS may be significantly better than by x-ray spectroscopy. It may therefore be possible to determine subtle changes in second messenger calcium in small subcellular compartments that could not be previously detected.

Application of the low-dose thickness measurement of glucose or ice-embedded protein crystals should improve the feasibility of high-resolution structure determination by means of electron crystallography.

PROPOSED COURSE: Improved techniques will be developed for extracting very small calcium and phosphorus signals from the energy loss spectrum; these developments will involve modelling the background spectrum to take plural scattering into account.

PUBLICATIONS: Leapman RD, Andrews SB. Characterization of biological macromolecules by combined mass-mapping and electron energy-loss spectroscopy. J Microscopy 1992;165:225-38.

Leapman RD, Andrews SB. Biological electron energy-loss spectroscopy: the present and the future. Microscopy Microanalysis Microstructures 1991;2:387-94.

Leapman RD, Hunt JA. Comparison of detection limits for EELS and EDXS. Microscopy Microanalysis Microstructures 1991;2:231-44.

Leapman RD. EELS quantitative analysis. In: Ahn C, Disko M, eds. Application of transmission EELS in materials science. Minerals Metals Materials Society, 1992;47-83.

Leapman RD, Hunt JA. Compositional mapping with electron energy-loss spectroscopy. Microscopy: The Key Research Tool 1992;22:39-49.

Leapman RD, Hunt JA. Compositional mapping by EELS. In: Bailey GW, ed. Proceedings of the 49th annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1991;8-9.

Batson PE, Leapman RD. Spatial resolution in electron energy-loss scattering. In: Bailey GW, ed. Proceedings of the 49th annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1991;474-5.

Leapman, RD. Limits of detectability for parallel electron energy loss spectroscopy. In: Electron microscopy and analysis 1991: Institute of Physics conference series. London: Institute of Physics, 1991;119:95-100.

Leapman RD, Newbury DE. Trace analysis of transition elements and rare earths by parallel EELS. Proceedings of the 50th annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1992;1250-1.

Leapman RD, Hunt JA, Buchanan RA, Andrews SB. Parallel-EELS mapping of calcium in cryosectioned cells. Proceedings of the 50th annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1992;1566-7.

Leapman RD, Hunt JA, Buchanan RA, Andrews SB. Measurement of low calcium concentrations in cryosectioned cells by parallel-EELS mapping. Ultramicroscopy (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10303-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Assessment of Scratching in Biliary Cirrhosis Patients

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas Talbot, M.S.	Mechanical Engineer	BEIP, NCRR
Joseph Schmitt, Ph.D.	Staff Fellow	BEIP, NCRR
Elijah Walker, M.S.	Section Chief, ACES	BEIP, NCRR

COOPERATING UNITS *(if any)*
LDS, NIDDK (N. Bergasa, E. A. Jones)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects _(b) Human tissues (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Patients with primary biliary cirrhosis suffer from severe bouts of itching, manifested by chronic scratching. In order to evaluate the efficacy of experimental drugs that may relieve the itching, it is necessary to quantify the scratching. A piezoelectric foil cemented to the patient's primary scratching fingernail will transduce mechanical vibrations generated by the scratching into an electrical signal, which is applied to the input of a miniature FM transmitter. After the signal is obtained with an FM receiver, it will be processed by a custom-designed frequency counter, which in turn is interrogated and reset periodically by a personal computer. The study will consist of measurement periods of six to eight hours, both before treatment and after treatment with specific drugs. Each patient will serve as his or her own control.

MAJOR FINDINGS: A scoring scheme was devised and implemented that provides an average scratching activity index per hour. This index was used in a large double-blind clinical protocol to determine the viability of Naloxone, an opiate receptor antagonist, as an ameliorating agent in the treatment of pruritus. The study, which is now complete, ran for four consecutive 24-hour periods, with two control (placebo) periods and two Naloxone periods. Neither the patients nor the investigators were aware of the contents of the I.V. infusate during the study. The results clearly demonstrated a statistically significant reduction in scratching activity while the Naloxone was being infused, indicating that the drug is effective in ameliorating the pruritus associated with cholestasis. The results of the study have been published. In addition, a new clinical protocol is underway, consisting of a double-blind study designed to investigate Nalmafene, the oral counterpart of Naloxone. Parallel analysis of certain underlying circadian rhythms that may unveil imprinted scratching behavior is also underway. Preliminary indications imply that scratching may be an independent biorhythm, as well as a manifestation of a dermal antagonist.

PROPOSED COURSE: Simultaneous recordings of ECG and scratching are being obtained to investigate the frequency of the biorhythms that may be associated with scratching.

PUBLICATIONS: Bergasa NV, Talbot TL, Alling DW, Schmitt JM, et al. A controlled trial of Naloxone infusions for the pruritus of chronic cholestasis. Gastroenterology 1992;102:544-9.

Talbot TL, Schmitt JM, Bergasa NV, Jones EA, Walker EC. Application of piezo film technology for the quantitative assessment of pruritus. Biomed Instrum Technol 1991;25:400-3.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10305-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
CC Image Management System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ronald L. Levin, Sc.D. Biomedical Engineer BEIP, NCRR

COOPERATING UNITS *(If any)*
MIS, CC (J. Foy, T. Lewis); DCRT

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In order to facilitate the viewing and analysis of biomedical images, the Clinical Center is in the process of designing and developing an Image Management System (IMS) to complement the current text-based Medical Information System (MIS). This new system will consist of the following four key components: (1) several different types of medical image gateways (MIGs); (2) an image archival and retrieval system (IARS) that has an MIS interface; (3) several different types of workstations (WS) possessing a common biomedical shell; and (4) an interconnecting communications network. The MIGs will permit digital radiological images (i.e., MR, CT, PET, etc.), nondigital radiological images (i.e., plane-film X ray), and nonradiological images (i.e., video-microscopic images, EKG tracings, patient monitor information, etc.) to be converted to a common format, compressed without loss, and automatically sent to the IARS. The IARS will permit not only the long-term archiving on optical media of the images coming from the MIGs, but also the retrieval of multiple modality images to local workstations in response to queries via the existing MIS data base, the new IMS data base, or individual departmental data bases. In the workstation area, we will be developing two or three different types of biomedical workstations. The key to this series of workstations is that they would utilize the same biomedical shell (i.e., user interface). Application programs for diagnostic imaging, stereotaxic surgical planning, radiation therapy planning, tumor staging, etc., will also be developed.

OBJECTIVES: To facilitate the exchange of medically related images by permitting images from multiple sources to be accessed via a single data base and in a single file format, and to facilitate the manipulation and comparison of medically related images together with text-based medical records through the use of a specially designed biomedical workstation.

PROPOSED COURSE: This project is waiting for finalization of system design parameters and budgetary approval. Phase I (testing of prototypes of the major system components) will be started in FY92 through the implementation of the Multimodality Radiological Image Processing System for the newly created Diagnostic Radiology Research Program, OD (Z01 RR 10339-03 BEI).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10307-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pulse Oximeter Calibrator

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joseph M. Schmitt, Ph.D.	Staff Fellow	BEIP, NCRR
Guanxiong Zhou	Visiting Scientist	BEIP, NCRR
Elijah C. Walker, M.S.	Section Chief, ACES	BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Over the last year, we have developed and demonstrated a new method for simulating the optical signals measured by pulse oximeters. Because it is easy to implement, the method should enable portable simulators to be built inexpensively for use in hospitals and factories. The Food and Drug Administration is now investigating if a simulator based on our method would be suitable for comparing pulse oximeters as part of a new standard for apnea monitoring.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To design and construct a prototype oximeter calibrator for use in the Clinical Center. Also, to develop general mathematical models and calibration techniques for comparing the performance of oximeters.

METHODS EMPLOYED: Using photon-diffusion theory, we first analyzed the effect of the primary variables affecting the calibration of pulse oximeters. Results clearly showed that under certain physiological conditions, measurement errors can exceed clinically acceptable bounds. Therefore, there is a need for better standardization among the various calibration approaches used by manufacturers of pulse oximeters. To help meet this need, we have been developing calibration systems that employ electronically controlled liquid-crystal light valves to simulate the signals measured by pulse oximeters. By adjusting the amplitude, waveshape, and frequency of the voltage driving the valve, its time-dependent transmittance can be made to resemble that of a blood-perfused finger. Our latest prototype, built using inexpensive optical components, has been successfully demonstrated.

SIGNIFICANCE: Many factors affect the accuracy and repeatability of oxygen saturation values measured by pulse oximeters, including the volume of blood in the target tissue and the spectral characteristics and configuration of the light sources in the optical probe. The results of our photon diffusion analysis have clarified the role of these factors. The calibration methods that we are developing can potentially simplify the factory calibration of pulse oximeters, and form the basis for an inexpensive device that can be used by clinical and biomedical maintenance personnel to verify proper operation of pulse oximeters in the hospital.

PROPOSED COURSE: We will continue to encourage the transfer of this technology to the Food and Drug Administration, which is working in collaboration with private companies to manufacture a pulse oximeter calibrator. No further technical development within the NIH is anticipated.

PUBLICATIONS: Schmitt JM. A simple photon-diffusion analysis of the effects of multiple scattering on pulse oximetry. IEEE Trans Biomed Eng 1992;38:1194-1203.

Zhou GX, Schmitt JM, Walker EC. An electro-optic simulator for pulse oximeters. Med Biol Eng & Comput (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10309-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Signal Conditioning and Data Acquisition System for Sleep Deprivation Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Courtney P. Mudd, Ph.D. Biomedical Engineer BEIP, NCRR

COOPERATING UNITS *(if any)*
CPB, NIMH (C. Everson)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An AT-type microcomputer with a resident 16-channel, 12-bit A/D board is used to measure and store signals from small (0.5 mm dia. x 3 mm long), fast (20 msec), glass-encapsulated thermistors. The calibration values for each thermistor are stored in a separate calibration file and are loaded into the program when the program is started. A thermistor bridge circuit accommodates a tolerance of ±20% in the nominal thermistor resistance, and covers a range of 25° to 45°C with 0.01°C resolution. Custom software controls the sample acquisition rate, filtering linearization, calibration, and storage of the signals. The A/D card runs in a "background" mode for 30-second intervals, with the data transferred directly to a specified memory location. This mode of operation frees the CPU for processing the previous interval's data and allows uninterrupted, or continuous, data acquisition. After the first cycle, the memory location is switched and another cycle is started. The program then downloads the 16-channel data from the previous memory location and determines the resistance of each thermistor from the voltage readings. From this resistance and the calibration values, the temperature of each thermistor is calculated. The "background" operation also allows updating or changing the calibration values (new thermistor) or relocating the thermistor (channel change) without interrupting the data acquisition.

OBJECTIVES: The processing and acquisition system have now been in operation for six months. During this evaluation period, we have determined that the thermistor measurement system has an accuracy of ±0.02°C with a resolution of 0.01°C. The transmitter system, which comes with no calibration or sensitivity information, has a resolution of 0.05°C, but an accuracy of only ±0.2°C. The source of this error was traced to the water bath calibration scheme used to measure the sensitivity of the transmitters.

SIGNIFICANCE: The operation of this continuous data acquisition system will allow uninterrupted, long-term investigations into the interdependence of diet and sleep. Preliminary studies have shown a definite relationship between brain temperature and sleep deprivation. An accurate system for measuring both the brain temperature and sleep state (vigilance) is indispensable for investigating this effect. Our brain temperature accuracy of ±0.02°C represents approximately a threefold improvement over the present system.

PROPOSED COURSE: The data acquisition software has been developed to handle a wide variety of anticipated errors without operator intervention. Since the data acquisition is continuous (24 hr/day), the software is designed to allow the computer to recover from a power interruption, determine where it should be in the data acquisition cycle, and restart the program at the correct time in the data acquisition cycle. We have also incorporated a feature that allows the operator to select certain epochs for complete (unprocessed) data download to a floppy disk for Fourier analysis of the fast signals (EEGs and EMGs). An automated, computer-controlled transmitter calibration system has been developed that has reduced the transmitter temperature error to ±0.05°C.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10310-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microcalorimeter Measurements of DNA-Protein Interactions

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Courtney P. Mudd, Ph.D. Biomedical Engineer BEIP, NCRR

COOPERATING UNITS *(if any)*
LMB, NIDDK (P. Ross); FCRF, NCI (Y. Tekeda); IR CH, NHLBI (R. Berger)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The use of various proteins to modify the human immune system response is of intense interest in the areas of organ transplants, AIDS, and chemotherapy. At the NCI, several new proteins have been developed for this purpose. At present, these proteins are available only in small amounts. By mixing these proteins with carefully constructed molecules of DNA and measuring the enthalpy, the protein-DNA bond interactions can be determined. Investigators at the NCI and NIDDK consulted the BEIP about measuring the heats of these small samples. We recommended using our tantalum stopped-flow microcalorimeter, which has the capability of measuring heats of 10 microjoules or less in 80-microliter samples. The first experiments showed that we could measure heats in the range of 5 microjoules with a standard error of less than 0.5 microjoules. We have now completed the preliminary study of this protein with two different DNA hosts at 25° and 37°C. Using the tantalum stopped-flow microcalorimeter, we were able to measure a binding heat difference when one base pair on the DNA host was changed. Results show that the enthalpy is very low, which indicates that the binding is almost exclusively entropy-driven.

OBJECTIVES: To reduce the priming volume from 1 ml to approximately 0.2 ml in order to further conserve reagents, and to provide a means of automatic loading for unattended operation. We have also found that part of the flow artifact is due to the pressure sensitivity of the sensors we use to detect the heat flow. The new instrument will incorporate a low-stress mounting system with no static pressure on the sensors.

SIGNIFICANCE: In studying these interactions, very often we change only one base pair in each experiment. Even a reasonably small DNA molecule can represent 30 to 40 experiments to study the protein's reactions. Automatic loading would allow unattended operation during nonworking hours. The reduced priming volume will conserve expensive reagents ($4,000 to $5,000 per experiment) by reducing the number of "lost" runs at the buffer/reagent flow interface. The reduction of the pressure-induced flow artifact will increase the usable resolution of the measurement. This is especially important since the measured heats are very low (10 to 20 microjoules).

PROPOSED COURSE: We have identified several rotary valves which can be computer-interfaced to sequence loading and running of the calorimeter. If we change the inlet lines to the calorimeter from 1.5 mm ID to 0.5 mm ID, the priming volume can be reduced by a factor of 9. The pre-equilibrator in the calorimeter must be redesigned, however, to ensure that the reagents are in thermal equilibrium with core temperature when they reach the mixer. We plan to use small-bore (0.5 mm ID) tantalum tubing for the inlet tubes to reduce the priming volume to approximately 200 microliters (vs. 1 ml in the old design). To reduce the pressure-induced flow artifact, special spacers will be used to standoff the flow tube from the sensors by 25 microns. The 25-micron space will be filled with heat sink compound to conduct the heat from the reagent tube to the sensors. The smaller inlet tubing will require a redesigning of the mixing chamber.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10313-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
High-Speed Multichannel Spectrophotometer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
John Cole, MSEE	Electrical Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(If any)*
LCB, NHLBI (R. Hendler); CSL, DCRT (H. Frederickson)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A number of commercial multichannel spectrophotometers using charge-coupled devices have recently been developed for general use in biomedical research. We have explored the possibility of improving both the speed and the accuracy of such instruments by exploiting other new devices.

OBJECTIVES: To develop a 100-channel spectrophotometer capable of sampling all channels at a maximum rate of 100,000 samples per second with 12-bit resolution.

METHODS EMPLOYED: A commercial spectrophotometer will disperse the light to two 50-element photodiode arrays. Each channel will integrate the signal between sample times, digitize it, and store it in a first-in, first-out register prior to digital multiplexing for transmission to a computer. The computer will provide full control over the sampling program, as well as over such experimental parameters as flow and flash photolysis; it will also handle all of the processing and permanent storage, will display data, and will perform various other functions.

SIGNIFICANCE: Achievement of the proposed specifications will open up a variety of new applications. The increased temporal resolution will permit investigation of early events in biochemical reaction kinetics that have been impossible to measure before. The ability to follow these processes with high accuracy and at multiple wavelengths enables investigators to track individual components within a complex reaction simultaneously.

PROPOSED COURSE: Design and construction are complete, and the instrument is in regular use. It is very close to meeting all original specifications, but additional testing and evaluation are still needed. We are exploring the possibility of cooling the diode arrays, adding a programmable offset to each channel, and increasing the gain to provide improved resolution. We are also looking into the possible application of image intensifiers to extend the short wave length response of the instrument. Other system components (such as the measuring light source) will be stabilized more tightly, and other optical refinments will be made.

An invention report has been filed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10314-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pulsed Photodynamic Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR
John W. Cole, MSEE	Electrical Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
ROB, DCT, NCI (A. Russo)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Experimental evidence indicates that the efficacy of photodynamic therapy (PDT) is temperature-dependent. This insight creates the possibility of using a temperature gradient in the tissue to compensate partially for the rapid attenuation of light, thus allowing effective treatment to a greater depth. By pulsing the surface temperature at an appropriate rate and illuminating only during the cool phase, the treatment will occur at a selected depth during the warm phase, due to the propagation delay experienced by the temperature wave.

OBJECTIVES: To develop a technique to provide effective PDT to a greater depth than is now possible.

METHODS EMPLOYED: Computer simulation of one-dimensional thermal diffusion has been carried out, using a SPICE program. The geometry is reasonable for the surface of the peritoneum and for slab-shaped organs (e.g., the liver). The surface temperature was approximated by a trapezoidal waveform, representing the temperature control that could be achieved by changing intralipid.

MAJOR FINDINGS: An empirical relationship was determined between the frequency of the temperature variation, the depth, the two thermal parameters of the tissue, and the amplitude and phase of the temperature waveform in tissue. This was correlated with analysis based on transmission line theory. In principle, the result allows determination of the frequency needed to optimize treatment at a specified depth; however, the attenuation was so severe that a useful elevation in temperature at interior points could not be achieved. The analysis was extended to include both linear and nonlinear effects of circulation. Even then, the increase in temperature at interior points relative to the surface, although greater, was not sufficient to be particularly useful. Considerable benefit might still accrue through secondary effects, such as modification of the oxygen delivery rate and modification of optical parameters.

SIGNIFICANCE: PDT is limited to treating superficial regions because of the rapid attenuation of light in tissue. This new approach has the potential to ease this limitation somewhat.

PROPOSED COURSE: The potential for exploiting secondary effects of temperature on circulation, and of circulation on oxygen availability, will be studied further. In addition, other methods of increasing the depth of treatment will be explored, including modification of the distribution profile of the photosensitive agent by ionophoresis, absorption, or other means; creation of a profile of an inhibitor, such as histidine or vitamin E; modification of the illumination schedule to exploit oxygen depletion; and use of pressure to inhibit oxygen replenishment near the surface. In addition, methods of improving light transmission through the skin will be explored, since hematoporphyrin derivative (HPD) apparently concentrates in skin, as well as in malignant tissue. Work on this project is expected to continue as new developments in inhibitors occur, and if there is a renewed attempt to apply PDT to breast cancer at a more sophisticated level.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10315-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Model of Magnetic Stimulation of a Nerve Fiber

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bradley J. Roth, Ph.D.	Staff Fellow	BEIP, NCRR
Peter Basser, Ph.D.	Staff Fellow	BEIP, NCRR

COOPERATING UNITS *(If any)*
Medical Neurology Branch, NINDS (L. Cohen, M. Hallett)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A model is presented to explain the physics of nerve stimulation by electromagnetic induction. Maxwell's equations predict the induced electric field distribution produced when a capacitor is discharged through a stimulating coil. A nonlinear cable model, containing active Hodgkin-Huxley elements, describes the response of the nerve fiber to this induced electric field. It is shown that the nerve fiber is stimulated by the gradient of the component of the induced electric field parallel to the fiber, which hyperpolarizes or depolarizes the membrane and may stimulate an action potential. Once the coil's position, orientation, and shape are given and the resistance, capacitance, and initial voltage of the stimulating coil are specified, this model predicts the resulting transmembrane potential of the fiber as a function of distance and time. Finally, it predicts complicated dynamics, such as action potential annihilation and dispersion. The model has been verified experimentally in humans. New coil designs for magnetic stimulation of peripheral nerves have been developed.

OBJECTIVES: To increase our understanding of the physics of magnetic stimulation, and to design improved coils, by developing a mathematical model of the interaction of a nerve fiber with the induced electric field.

SIGNIFICANCE: Although the electric field induced in an arm can be calculated accurately, its interaction with a peripheral nerve had not been elucidated. Basic questions such as the location of stimulation must be resolved before magnetic stimulation can be utilized fully as a clinical tool. This model provides insight into the interaction of the electric field with the nerve, and provides a tool for predicting new coil designs.

MAJOR FINDINGS: The magnitude and time course of the gradient of the component of the induced electric field parallel to the nerve fiber determines if and where stimulation occurs. A four-leaf coil design allows focal stimulation of a peripheral nerve. The location of the virtual cathode is sensitive to coil placement. A secondary virtual cathode and anode pair are predicted to exist for a circular coil.

PROPOSED COURSE: The model is being tested experimentally in humans. A new coil design is also being evaluated, using both *in vitro* and *in vivo* measurements. The model is also being simplified to improve its clinical utility.

PUBLICATIONS:

Panizza M, Nilsson J, Roth B, Basser P, Hallett M. Relevance of stimulus duration for activation of motor and sensory fibers: implications for the study of H-reflexes and magnetic stimulation. Electroenceph Clin Neurophysiol 1991;85:22-9.

Nilsson J, Panizza M, Roth B, Basser P, Cohen L, Caruso G, Hallett M. Determining the site of stimulation during magnetic stimulation of a peripheral nerve. Electroenceph Clin Neurophysiol (in press).

Basser P, Wijesinghe R, Roth B. The activating function for magnetic stimulation from a three-dimensional volume conductor model. IEEE Trans Biomed Eng (in press).

Basser P. Focal magnetic stimulation of an axon. IEEE Trans Biomed Eng (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10316-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Calculation of Electric Fields During Magnetic Stimulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bradley J. Roth, Ph.D.	Staff Fellow	BEIP, NCRR
Walter S. Friauf, MEE	Chief, EEES	BEIP, NCRR
Joshua Saypol	Student Researcher	BEIP, NCRR

COOPERATING UNITS *(if any)*
Medical Neurology Branch, NINDS (L. Cohen, M. Hallett)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Magnetic stimulation is a new technique for activating neurons that is both painless and noninvasive. Stimulation occurs when a brief current pulse is passed through a coil placed near the neuron, producing an electric field by electromagnetic induction. The goal of this project is to develop mathematical models to calculate the electric field induced in the human body during magnetic stimulation. Two models are developed in detail: (1) a model of the stimulation of a peripheral nerve in the arm, and (2) a model of the stimulation of the brain.

OBJECTIVES: To calculate the electric field distribution in the body during magnetic stimulation.

SIGNIFICANCE: Although the use of magnetic stimulation is growing rapidly, the technique has been applied clinically without a complete theoretical understanding of the induced electric field distribution. If this distribution can be calculated numerically, then some theoretical insight will be provided to guide future applications of the technique. In addition, these calculations will aid in the development of more focal coil geometries.

MAJOR FINDINGS: During magnetic stimulation, a charge distribution arises on the tissue-air surface that partially shields the interior of the body from the stimulus. It is essential to take into account the presence of this charge distribution to calculate the electric field correctly. Generally, this shielding effect is more pronounced for coils oriented perpendicularly to the tissue-air surface. The peak electric field occurs near the surface of the tissue.

PROPOSED COURSE: The present calculations all use a simplified geometry: a cylinder for the arm, and a three-sphere model for the brain. To determine the electric field in the tissue accurately, future calculations will be performed using a finite element model that can account for the exact geometry of the tissue. The results of these calculations will be compared to experimental data obtained by collaborators at the Clinical Center. Techniques are being developed to perform the inverse problem: Given a desired electric field in the head, what is the coil geometry required to produce that field?

PUBLICATIONS:

Saypol J, Roth B, Cohen L, Hallett M. A theoretical comparison of electric and magnetic stimulation of the brain. Ann Biomed Eng 1991;19:317-28.

Brasil-Neto J, Cohen L, Panizza M, Nilsson J, Roth B, Hallett M. Optimal focal transcranial magnetic activation of the human motor cortex: effects of coil orientation, shape of the induced current pulse, and stimulus intensity. J Clin Neurophysiol 1992;9:132-6.

Cohen L, Roth B, Wassermann E, Topka H, Fuhr P, Schultz J, Hallett M. Magnetic stimulation of the human cerebral cortex: an indication of reorganization in motor pathways in certain pathological conditions. J Clin Neurophysiol 1991;8:56-65.

Roth B, Cohen L, Hallett M. The electric field induced during magnetic stimulation. Electroenceph Clin Neurophysiol 1991;suppl 43:268-78.

Cohen L, Bandinelli S, Topka H, Fuhr P, Roth B, Hallett M. Topographic maps of human motor cortex in normal and pathological conditions: mirror movements, amputations, and spinal cord injuries. Electroenceph Clin Neurophysiol 1991;suppl 43:36-50.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10318-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Apple Macintosh II-Based Image Processing Workstation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Michael Unser, Ph.D. Visiting Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Personal computers such as the Macintosh II offer very cost-effective hardware configurations for simple image processing tasks. However, their programming using conventional techniques is notoriously difficult, due to the high level of sophistication of the user interface. The main purpose of this project was to devise powerful development tools to facilitate the development of specialized image processing software for such workstations. The first step was to create a simple image processing shell that is easy to modify for creating specialized applications; the main feature is that all complex interactions with the user interface are handled automatically, and that the programmer need only be concerned with the application-specific procedures. The second aspect was to develop a comprehensive set of basic image processing modules. At present, our software library includes about 250 subroutines that perform image processing tasks such as filtering, multiresolution and wavelet decompositions, edge detection, point transformations, and binary operations.

OBJECTIVES: To develop an extensive software library to facilitate and standardize the development of user-friendly image processing programs on the Macintosh II computer family.

METHODS EMPLOYED: Most software has been written in Absoft FORTRAN V2.4, and more recently in C using Think C V5.0. The integration of basic subroutines into application-specific programs is centered around the use of the FACEIT shell, which substantially accelerates and simplifies the development process. Our image processing software library currently includes a relatively large selection of point operators (histogram, nonlinear transformations, simple arithmetics, statistics, etc.), neighborhood operators (moving average and Gaussian smoothers, Laplacian, convolution, median and morphological filters, adaptive least squares and B-spline filters), wavelet transforms and image pyramids, and binary operations (automatic thresholding, thinning, expansion, erosion). There is also a collection of input/output subroutines for reading and writing images in TIFF format, display, and interactive specification of points, lines or regions of interest.

MAJOR FINDINGS: This new programming environment has cut down development time substantially and improved reliability. Special-purpose image processing software that was developed based on this strategy includes Mecho (automated extraction of serial myocardial borders from M-mode echocardiograms), GelFit (analysis of two-dimensional gels in a size-mobility map), μEdge (a comprehensive set of edge-detection algorithms), and μTongue (detection of the tongue surface from ultrasound images). FLIPS (Flexible Language-based Image Processing System) and MULTIMAGE integrate most of the currently available subroutines.

SIGNIFICANCE: The availability of efficient development tools is essential in setting up small collaborative projects with other researchers in the NIH community. There are several advantages in designing an image processing system based on a Macintosh II computer. First, this type of computer is now widely available for a variety of personal computing tasks, which means that the purchase of new equipment is usually not necessary. Second, the Macintosh II stands as a very cost-effective alternative to conventional image processing systems. Third, all Macintosh programs are designed to be very user-friendly, which makes them easy to use and facilitates their dissemination. Finally, most data formats are standardized, which greatly facilities the exchange of information among different applications. Custom programs and general-purpose applications (statistics, graphs, etc.) can be combined very efficiently to solve a specific problem.

PROPOSED COURSE: We will extend the present environment by adding new image processing functions. We are also planning to translate some of our subroutines in C for software development in a UNIX environment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10322-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Data Acquisition System for an Ultrahigh-Resolution Dedicated STEM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Carol R. Swyt, M.S. Physical Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
CAC, NIST (C. Fiori, R. Myklebust)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	3.0	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects _(b) Human tissues X (c) Neither
_(a1) Minors
_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An automated data acquisition system is being developed for the Vacuum Generators HB 501 ultrahigh-resolution dedicated scanning transmission electron microscope. The new analytical capabilities of this class of electron microscope require fresh approaches to computerization of data acquisition and analysis. Available commercial systems have been limited in both the kinds of data that can be acquired concurrently and the algorithms for processing the data. As part of this system, a desktop data acquisition and analysis program, called Desk Top Spectrum Analyzer and X-Ray Database (DTSA), has been developed for the Macintosh II (and higher) class of computers. The data acquisition is accomplished utilizing specially modified commercial plug-in bus cards and control software routines that interface to the control and analytical software of DTSA. A patent, to be held jointly by the NIH and NIST, is pending on this analytical tool. The DTSA is available from the Office of Standard Reference Data (OSRD), National Institute of Standards and Technology, for a modest fee. A copyright has been obtained by the OSRD.

METHODS EMPLOYED: The software is written in MPW Pascal. The user interface utilizes the full range of Macintosh utilities, including windows, menus, dialogs, and color display. Algorithms for all of the relevant physics for the generation of x-rays in a thin or bulk specimen by an electron beam, and detection by a lithium-drifted silicon detector, have been incorporated into the program. The user may select from a number of the most widely-used cross sections for x-ray generation, and may input microscope, specimen, and detector characteristics that determine the final spectrum distribution. Any of the functions calculated, such as the continuum distribution and characteristic peaks before and after convolution with the detector function, may be displayed in one of the ten 8192 real-valued overlaid "spectrum displays." The information in the x-ray database is available to the user via a menu which provides relative intensities with the lines, edges, or satellites specified in the user-selected units of eV, mm, Å, or (for wavelength dispersive x-ray spectrometry) sin(θ) for the crystal spectrometer chosen. Both linear and nonlinear spectral peak unravelling methods are available, and quantitation schemes for thin and bulk biological, geological, and other specimens have been provided. X-ray spectra acquired by wavelength dispersive x-ray spectrometry may be imported, converted to energy display, and processed using any algorithm in DTSA. Among the other routines provided are qualitative analysis, spectrum calibration, beam energy calculation based on the Duane-Hunt limit, peak stripping, background subtraction, Fourier transforms, spectral smoothing, and simple scaling and summing of distributions. Additionally, x-ray spectral data acquired with other systems may be transferred to the program and converted to internal format for processing. Any displayed distribution may be sent directly to a printer or stored in a file. The results of spectral unravelling and quantitation may be reported in any one of the common spreadsheet formats for statistical analysis and plotting.

An interface to the acquisition control software is provided in the program. The parameters for acquisition (such as data collection time, characteristics of the detector system, etc.) are supplied via the DTSA program to the acquisition software. Information such as dead time, real time, and data input rate are constantly displayed. As data are acquired, they are transferred to the Macintosh memory, and are available for constantly updated display as a spectrum on the Macintosh screen, and for processing with any algorithm in the DTSA program.

Version 1 of the program was released in October 1991 by the Office of Standard Reference Data, National Institute of Standards and Technology. A number of commercial x-ray analyzer manufacturers have negotiated to offer DTSA as an option with their systems.

SIGNIFICANCE: Though designed for the EBIMS VG HB501 STEM, the DTSA provides analysts in any laboratory with an easily customized means of obtaining x-ray data, spectrum or image, from any

electron microscope equipped with the appropriate detectors at about one-tenth the cost of traditional energy dispersive x-ray systems. In addition, it provides the full capabilities for data analysis developed over the years in the EBIMS group, and for data presentation and statistical analysis developed for the Macintosh computer. These capabilities are implemented in the typically user-friendly Macintosh environment. The program provides the analyst with a set of tools to simulate, analyze, and better understand the generation and detection of x-rays from his/her sample, and therefore to design more informative and efficient experiments. This can be done on the desktop, freeing the electron-beam instrument for productive data acquisition.

PROPOSED COURSE: Modifications will be made in the equations to provide capabilities for x-ray fluorescence data similar to those now provided for electron beam x-ray data. Image acquisition and processing routines, as well as wavelength dispersive x-ray spectrum and image acquisition and analysis capabilities, are being developed.

Negotiations are under way between commercial manufacturers of electron microscopes and x-ray analytical equipment, and the NIST and the NIH, for the rights to distribute and otherwise use the program.

PUBLICATIONS: Fiori C, Swyt C. A commentary on recent developments in x-ray detectors and ancillary instrumentation and data extraction algorithms. In: Peachey LD and Williams DB, eds. Electron microscopy 1990, vol 2: analytical sciences. San Francisco: San Francisco Press, 106-7.

Swyt CR, Fiori CE. Desktop generation and analysis of spectra. In: Peachey LD and Williams DB, eds. Electron microscopy 1990, vol 2: analytical sciences. San Francisco: San Francisco Press, 108-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10324-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
In Vitro Hemodynamic Models for Cardiovascular Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert J. Lutz, Ph.D.	Chemical Engineer	BEIP, NCRR
Travis Jones, Ph.D.	Chemical Engineer	BEIP, NCRR

COOPERATING UNITS *(if any)*
IR CB, NHLBI (J. Peacock); ROB, DCT, NCI (A. Epstein)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
3.0	2.0	1.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Physical models of vascular systems are being used extensively in our laboratory to study a variety of hemodynamic phenomena. During the past year, the major effort has been directed to projects in the following two categories: hemodynamic studies related to the development of turbulence during pulsatile flow, and intra-arterial infusions of radiation-sensitizing drugs into the uterine artery for the treatment of cervical cancer. (1) The onset of turbulence during oscillating flow has been studied in a series of straight, rigid tubes. An empirical correlation was developed that would predict the transition from laminar to turbulent flow as a function of the peak flow (Reynolds number), the frequency of oscillation (Womersley parameter), and the stroke volume (Strouhal number). Similar experiments were performed with branched tubes of two different area ratios, in models of pig coronaries, and in models of stenoses. Turbulence correlations, friction factor calculations, and velocity profile data were derived for these configurations. (2) A glass model of the human cervical artery system was fabricated, based on anatomical illustrations of the geometry. The model has been connected to a flow circuit, and the proper flow rates and wave forms for ileofemoral and cervical flows have been established. The system is being prepared for studies of dye injections into the various branch arteries via intra-arterial catheter, to assess the distribution of injectate into the various arterial regions both visually and quantitatively.

OBJECTIVES: (1) The objective of the turbulence studies was to determine what conditions of total flow, frequency of flow oscillation, and stroke volume would cause a transition of the flow from laminar to turbulent flow in simple, straight tubes. This information is relevant to gaining a better understanding of the flow conditions that could lead to turbulence in the more complicated geometry of the coronary vasculature. The presence of turbulence in the coronary circulation is suspected as a cause of thrombus formation around coronary artery stents that have been inserted for structural support following angioplasty. Another objective was to determine the effect of branching in the flow on the generation of flow instabilities and turbulence. Two simple branching configurations were constructed as a 45° branch, with parent-to-daughter area ratios of 1 to 2 and 1 to 1.15. These latter results were compared to data collected in a casted model of a pig coronary artery. Models of 75% and 90% stenoses in straight tubes were studied to determine the onset of turbulence in pulsatile flow conditions, as simple simulations of coronary restenosis. The data for these various models have been used to develop correlations that predict the onset of turbulence and correlations for estimating friction factors. Velocity data has also been generated and compared to theory over the range from laminar through transition to turbulent flow.

(2) Intra-arterial administration of drug solutions can provide a regional advantage to therapy by yielding a high local concentration of drug in a tumor region while maintaining low, less toxic systemic levels. Radiation-sensitizing drugs have been used with good success, in combination with radiation, in treating gynecological cancers. To achieve optimum success in treating cervical cancer, the radiosensitizing drug would be delivered only to the tumor-bearing region, and would avoid other sensitive tissues around the uterus and rectum. *In vitro* flow models of the cervical artery network will allow us to study proper placement of infusion catheters in the uterine artery, and assess different methods of drug delivery that will insure adequate distribution of the drug to the tumor site alone, and avoid unwanted regions.

METHODS EMPLOYED: (1) Straight-tube and branched-tube models were constructed from glass tubing or were fabricated by molding clear silicone rubber (RTV-615) around a low-melting alloy (Cerrosafe) that was subsequently melted out. A pig coronary model was made by injecting molten Cerrosafe through the aortic branch of an excised pig heart, digesting away the tissue, and casting a silicone rubber mold around the Cerrosafe, as explained above. Four stenoses models were made by boring holes axially through plexiglass cylindrical plugs. Two models were nonsymmetrical stenoses of 75% and 90% area reduction, and two models were axisymmetric area reductions of 75% and 90%. These plugs were inserted snugly into the straight-tube model. In these models the onset of turbulence was detected with hot film and with electrochemical probes that measured wall velocity gradients (shear stress). The probes were mounted flush with the inner wall of the tubes or coronary arteries at various axial locations.

Velocity measurements were made using a laser Doppler system. Oscillatory flow was generated through the models by using a combination of a steady flow pump and a Scotch yoke with variable frequency and amplitude capability. Analog signals from the measuring probes and the laser were recorded by computer, and the transition from laminar to turbulent flow was determined by the initial appearance of irregular disturbances in the signals.

(2) A flow model of the ileofemoral arterial network of a human, including branches of the uterine and cervical arteries, were fabricated from glass using published anatomical illustrations as a guide. The model system is connected to a flow system that generates pulsatile flow waves. The branches of the cervical and uterine artery models are connected to a collection device that allows individual sampling of the effluent fluid (simulated blood) from these branches. A red dye will be injected via a catheter at various locations in the arterial system, and fluid will be sampled from the distal branches to determine the distribution of dye by measuring dye concentration spectophotometrically. Various injection methods will be tested, and several novel catheters may be investigated. Flow visualization studies will be recorded on videotape to assess if any streaming of the infused solutions occurs during the various tests.

MAJOR FINDINGS: (1) The presence of oscillations in the flow was found to retard the onset of turbulence at a given average flow rate. An empirical correlation was derived from dimensional analysis that related the peak Reynolds number (Rep) at which turbulence would commence to the Womersley parameter (Alpha) and the Strouhal number(St) by a power law function:

$$Re_p = (K)\ (Alpha^{\ a})(St^{\ b})$$

The constants K, a, and b were determined by curve-fitting the turbulence data. Flow visualization studies showed the instabilities as small eddies that propagated in the flow, especially in the branched tubes near the bifurcation regions. The correlations for the branched tubes were similar to that for the straight tube, with similar values for exponents a and b, but with a lower value for K; that is, the turbulence occurred at lower values of peak Reynolds number for the branched tubes, but had the same functional dependence on Alpha and Strouhal numbers as the straight tubes. Identical correlations were obtained for branched tubes of area ratio 1 to 1, as well as for 1 to 1.15. Turbulence occurred in the left descending coronary artery (LAD) of the pig model in a similar fashion as in the simple 45° branch model. However, for the left circumflex coronary artery (LCX), turbulence appeared to occur at slightly lower peak Reynolds numbers than in the LAD or in the 45° branches. This may be a function of this particular pig coronary model. The placement of wire stents in the coronary model caused a transition to turbulent flow when the cardiac output was double the resting condition, simulating mild exercise. At resting cardiac output, flow was laminar with and without stents. At moderate exercise cardiac output flow rate

(three times resting), flow in the coronaries appeared turbulent even without the stents in place. The data from the stenoses model studies indicate that at Reynolds numbers for flow that represent resting cardiac output flow conditions, the flow inside the stenoses remains laminar, but the shear stresses may be high enough to cause platelet aggregation and thrombosis.

(2) Construction of a cervical model and flow system has been completed. Appropriate blood flow rates and flow wave forms have been determined from published literature values, and proper methods to achieve these flow rates in the model have been devised. Standard radiology catheters (Cobra, Medi-tech) have been positioned in the cervical artery models. Data will determine the proper catheter placement to localize the infused solution to a particular desired region of the cervix where a tumor may exist. New catheters and injection techniques will be studied that could eliminate streaming and improper distribution of drugs.

SIGNIFICANCE: (1) *In vitro* flow models are useful in studying flow characteristics of coronary arteries. In pulsatile flow, oscillations seem to stabilize flow and retard turbulence. Mild exercise may cause turbulence in coronary arteries after stent deployment.

(2) To achieve maximum therapeutic benefit with minimum toxicity to the patient, proper delivery of drug solution to the desired tumor-bearing region must be achieved. *In vitro* flow models are very useful in assessing delivery methodologies (e.g., catheter placement, catheter design, and injection procedures).

PUBLICATIONS: Froelich JL, Lutz RJ, Barth KH. Infusate distribution from various thrombolysis catheters--*in vitro* simulation. In: Proceedings of the 17th annual meeting of the Society of Cardiovascular and Interventional Radiology. Washington, D.C.: Society of Cardiovascular and Interventional Radiology, 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10327-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mass Mapping of Macromolecular Assemblies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Richard D. Leapman, Ph.D. Physical Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
LN, NINDS (S. B. Andrews, T. Reese, P. Gallant); LSBR, NIAMS (A. Steven, F. Booy); Lehigh Univ. (J. Hunt)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The VG Microscopes HB501 field-emission STEM has been used to record low-dose digital annular dark-field mass maps of isolated, rapidly frozen protein macromolecular assemblies. Data were recorded from microtubules complexed with the cytoplasmic motor protein, kinesin. First, the molecular weight of free kinesin molecules (~380±15 kDa) was established by calibrating the digital micrographs with tobacco mosaic virus particles and with the 13-protofilament microtubules. Then, the masses of the attached kinesins were measured in order to characterize how they interact with the microtubule. The results show the first direct evidence of cross-bridging of microtubules by single kinesin molecules, and indicate that kinesins bind to microtubules not only by their heavy chains in the head domain, but also by a light-chain binding site in the foot. Preliminary molecular weight data were also obtained from an important phosphorylation protein, CAM kinase II, found in the postsynaptic density of the brain.

OBJECTIVES: To measure molecular weights of isolated macromolecular assemblies in the high-resolution scanning transmission electron microscope (STEM) by using the low-dose elastic dark-field imaging technique.

METHODS EMPLOYED: Samples were prepared by plunge-freezing suspensions of macromolecules adsorbed onto thin (~3nm) carbon support films, along with tobacco mosaic virus as an internal standard. The molecules were freeze-dried in the VG Microscopes HB501 STEM after cryotransfer at liquid nitrogen temperature. Mass mapping was performed at low dose with single-electron sensitivity by means of a PC486-based digital acquisition. The digitized images were analyzed using the IMAGE program on a Macintosh II computer.

MAJOR FINDINGS: Images of individual kinesins appeared as asymmetrical dumbbell-shaped structures, approximately 36 nm long, with an average molecular weight of 380±15 kDa. The kinesins could be segmented into three domains. The larger end (the head) was spherical, and measured 14 nm in diameter. This piece, along with the thin central stalk, had a mass consistent with a coiled alpha helix, and could be accounted for by the combined mass of two heavy chains. The smaller end (the foot) was consistent with the mass of the two light chains. On the microtubule bundles, there were frequent bridging structures, and image analysis gave a molecular mass that was indistinguishable from that of single isolated kinesins. There were also many sidearms that had the same mass as that of isolated kinesins, and further analysis showed that the foot, as well as the head, could be attached to the microtubules.

Preliminary data recorded from the enzyme CAM kinase II were consistent with that of an "eight-petal flower" arrangement of subunits, which may be relevant to its autophosphorylation behavior.

SIGNIFICANCE: The kinesins are a family of cytoplasmic motors that function to transport organelles along microtubules; in brain, they are involved in anterograde transport of vesicles down the axon. In addition to this tightly-bound kinesin, there is also a large pool of cytoplasmic kinesin, the function of which is unknown. The results of the STEM mass mapping suggest that kinesin could also serve the important role of providing a motor for microtubule-microtubule movements.

PROPOSED COURSE: It is planned to measure the masses of other proteins, including molecularly engineered kinesins that exhibit different motor functions, neurofilament subunits, and viral proteins.

PUBLICATIONS: Andrews SB, Leapman RD, Gallant PE, Reese TS. Characterization of microtubules by dark-field STEM and replication. In: Bailey GW, ed. Proceedings of the 49th annual

meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1991;152-3.

Andrews SB, Gallant PE, Leapman RD, Reese TS, Schnapp BJ. Single kinesin molecules cross-bridge microtubules *in vitro.* In: Proc Natl Acad Sci USA (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10329-04 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Optimized Mammography Instrument

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Alec Eidsath, Ph.D. Staff Fellow MES, BEIP, NCRR

COOPERATING UNITS *(If any)*
NCDRH, FDA (R. Jennings); DR, CC (J. Vucich)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects _(b) Human tissues (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Mammography plays a vital part in the early detection of breast cancer. Unfortunately, mammography units being used today have never been analyzed from a systems point of view. Multiparameter optimization studies of a mammography system have been reported by Muntz et al. (Med Phys **12**, 5, 1985) in which the patient dose has been minimized while maintaining satisfactory image quality. The most important new feature of the design is the presence of an array of thin metal vanes arranged in an arc about the x-ray source, between the breast and the film. The vanes absorb scattered radiation, and thus improve the quality of the image. In order for the vanes not to image on the film, the grid must move in a precisely controlled profile during the exposure.

OBJECTIVES: To develop an improved antiscatter device for use in mammography.

METHODS EMPLOYED: Advanced composite materials will be used in the design of parts of the device, thus providing both high strength and low x-ray absorbance. The motion will be controlled using a stepping motor with programmable motion profiles. Sensing of the position of the grid will be accomplished using high-speed fiber optic sensors.

SIGNIFICANCE: This topic has received a great deal of attention in the press recently, and regular mammograms are now recommended for all women over the age of 40. Since more mammograms will be taken, it is extremely important to reduce the dose to the patient in order to lessen any possible problems caused by radiation. The new instrument has a theoretical dose reduction factor of three.

PROPOSED COURSE: The moving grid assembly has been designed and constructed. It has spent the last year at the FDA, undergoing tests on the transmission of radiation. The assembly as constructed reduces the radiation dose to the patient by a factor somewhat greater than two. In July it was sent to Fischer Imaging in Colorado, to be installed on a slightly modified mammography unit. It will then be returned for experimental trials here at the NIH. If the trials are successful, the new instrument will be commercialized by Fischer.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10331-03 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Microanalysis of Rapidly Frozen Tissue in the Field-Emission STEM

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	BEIP, NCRR
Shanling Shi, M.S.	Visiting Associate	BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	BEIP, NCRR
Carol Swyt, M.S.	Physical Scientist	BEIP, NCRR

COOPERATING UNITS *(If any)*
LN, NINDS (S. B. Andrews, T. Reese, R. Buchanan); Case Western Reserve Univ. Sch. of Med. (D. Landis)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Measurements of ion concentrations were obtained from synaptic structures in the molecular layer of mouse cerebellar cortex in order to determine physiological changes that occur after neuronal activity. The areas of interest were first characterized by low-dose, dark-field mapping, from which the dry mass content could be determined. Microanalytical data could subsequently be corrected for mass-loss produced by electron beam damage. X-ray spectra, recorded from postsynaptic endoplasmic reticulum (ER) cisterns situated in Purkinje cell dendritic spines, showed two different populations: one with high calcium levels in the ER and low levels in the surrounding cytoplasm, and the other with lower calcium levels in the ER and higher levels in the cytoplasm. Additional analyses were performed on the dendrite mitochondria and ER, as well as on presynaptic terminals and neuroglia.

OBJECTIVES: To determine changes that occur in ionic composition of postsynaptic terminals in cerebellar cortex during neuronal activity. Of particular interest is the composition of membrane-bound structures of endoplasmic reticulum that are believed to regulate calcium during synaptic activity.

METHODS EMPLOYED: Rapidly frozen, 100-nm-thick cryosections of mouse cerebellar cortex were cryotransferred into a VG HB501 STEM and freeze-dried *in situ*. Digital low-dose dark-field images were first acquired from selected areas of the sections in order to characterize the structures of interest. It was necessary to correct the dark-field intensities for nonlinearity of the signal; this correction was achieved by using EELS to determine the thickness of the carbon support film in terms of the inelastic mean free path. X-ray spectra were then obtained with an ultrathin window energy-dispersive spectrometer subtending a solid angle of 0.18 steradians; data were quantified using the DTSA software on the Macintosh II computer.

MAJOR FINDINGS: We have obtained low-dose, dark-field images of truly ultrathin cryosections of directly frozen tissues. The advantages of this new technique include: (i) previously unattainable views of intracellular organization in unfixed tissues; (ii) the use of elastic scattering to measure directly the relative dry mass content of undamaged intracellular organelles and compartments; and (iii) the use of this dark-field measure of organelle mass to correct continuum-derived mass estimates during energy-dispersive x-ray spectroscopic (EDXS) microanalysis. It has now become possible to recognize synaptic structures unambiguously and to obtain EDXS spectra with large numbers of counts, thus greatly improving the microanalytical sensitivity to the sub-millimolar (dry weight of tissue) range. Data show two populations of denditic spines, one with relatively high (>5mmol/kg) calcium concentrations in the ER cisterns correlated with low cytoplasmic calcium (<2mmol/kg), and the other population with low calcium in the ER cisterns correlated with high calcium in the cytoplasm.

SIGNIFICANCE: For the first time, it is possible to answer important questions unambiguously about compositional changes that occur during brain activity. These advances have been brought about recently by the improvement in cryosectioning techniques and efficient low-dose STEM imaging techniques, as well as by the high performance of the field emission STEM for obtaining statistically useful data from very small volumes of the specimen.

PROPOSED COURSE: It is planned to continue to obtain further x-ray data from rapidly frozen, cryosectioned brain tissue, and to address questions concerning the role of calcium in synaptic activity.

PUBLICATIONS: Andrews SB, Buchanan RA, O'Connell MF, Leapman RD. Quantitative mass analysis of cryosections in the field-emission STEM. In: Proceedings of the 50th annual meeting of the Electron Microscopy Society of America, 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10335-03 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Design and Implementation of an Equipment Management Program

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Elijah C. Walker, M.S.	Section Chief	ACES, BEIP, NCRR
Thomas L. Talbot, M.S.	Mechanical Engineer	ACES, BEIP, NCRR
Joseph Bucolo	Electronics Technician	ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*
OD, CC (D. Cirelli, L. Eldridge)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section, CCIS

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In collaboration with the Clinical Center, ACES/CCIS is developing an equipment management program to enhance clinical research and patient care by providing a comprehensive program for the management of clinical and nonclinical electrical equipment. The overall goals of the program are to assure the safety and performance of clinical equipment; to assure compliance with federal, JCAHO, and other safety/performance standards; to maximize availability of equipment to designated patient care areas; and to manage costs. The Equipment Management Program will be administered by the Clinical Center, and involves policies and procedures affecting ACES (safety and standardization), CCIS (maintenance, inventory management, and prepurchase planning), and all Clinical Center/Institute clinical equipment users. Specific policies and procedures are being developed to address inventory control; equipment repair; periodic inspections; preventive maintenance; prepurchase planning; user education; equipment distribution, storage, and disposal; recalls; incident reporting; and alerts. A comprehensive manual will serve to document all policies and procedures pertaining to equipment management. A computer-based inventory management system is currently being implemented.

PROGRESS: A CCIS administrative manual has been written that addresses the quality assurance and risk management policies of the Clinical Center, the Joint Commission on Accreditation of Healthcare Organizations, and the BEIP.

Comprehensive policies have also been written that affect all areas of the hospital complex (Building 10) involving patient care equipment. These policies are under final review, and are expected to be implemented this fiscal year. A PC-based (AIMS software) computer system is operational, with increased staff usage. Macintosh computers are currently being networked (via Ethernet) into the existing system. New functional tests are continually being written, concurrent with taking a complete inventory of all patient-care equipment.

PROPOSED COURSE: Specific quality indicators are being developed for reporting to the CC Safety Committee and JCAHO. New hospital equipment management policy has to be implemented.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10336-03 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Kinetics of Folate Metabolism

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Paul F. Morrison, Ph.D. Physical Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
Medicine Branch, NCI (C. Allegra, D. Boarman)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.13	0.13	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent observations of folate pool dynamics in cells treated with trimetrexate or methotrexate have revealed different folate depletion responses in breast and colon cancer cell lines than in hepatoma lines. An explanation of this difference is being sought in terms of the biochemical kinetics of the folate cycle. A folate cycle biochemical network model has been employed to predict that the enzyme activity differences known to exist across several colon cancer lines are sufficient to cause the diverse patterns of folate depletion observed. Cells with both ten- and fortyfold elevations in thymidylate synthase (TS) activity were predicted to exhibit nearly complete folate depletion when exposed to 1-μM doses of methotrexate (MTX). Experiments have been completed in the Medicine Branch, NCI, on two cell lines selected for these increased levels of activity (plus controls) in which the cells were exposed to both 1-μM and 10-μM doses of MTX. Comparisons of the human colon line data with theoretical estimates based largely on human breast line parameters showed agreement at all nonzero doses and TS activities. Agreement, however, was lacking in the cell line with normal TS activity exposed to the higher MTX dose; theory predicted a short-term increase in 10-formyl tetrahydrofolate, while experimentation showed a rapid decrease. Possibly, this disparity is due to an overrepresentation of the sensitivity of TS to MTX polyglutamate inhibition, an effect more noticeable at larger drug doses.

RELEVANT BEIP PROJECTS: 85-136, Kinetics of Folate Metabolism.

OBJECTIVES: To develop an understanding of the fundamental biochemical interactions between the normal constituents of the folate cycle and various drugs active against elements of this cycle. To use this knowledge to optimize or develop drug protocols aimed at killing cancer cells.

METHODS EMPLOYED: A biochemical kinetic model of the folate cycle in human cancer cells, originally developed and calibrated against experimental culture data to describe breast cancer cells, was employed to describe the approximate behavior of colon cancer lines. Three colon lines were modelled: one parent line and two sublines selected by growth in 5-fluorouracil for ten- and fortyfold higher TS activities. The unperturbed parent line did not exhibit substantial differences in folate pools from the MCF-7 breast cancer line; therefore, Vmaxs and Michaelis constants were left at their MCF-7 values. Thymidylate synthase (TS) activities (Vmax) were increased to model the resistant sublines, with the model predicting substantial depletion of reduced folates in these lines after 5 hours of exposure to MTX and nearly complete depletion after 20 hours of exposure. Predicted depletion percentages were then tested against folate depletions observed experimentally in the three colon cancer lines after exposure to 1-μM and 10-μM doses of MTX. Folate pools were determined by reverse phase HPLC following cleavage of the polyglutamate tails.

MAJOR FINDINGS: The major finding was that increased TS activity alone could account for most of the folate depletion observed in 5-FU resistant colon cancer cells. Experimental determinations of pool sizes in the elevated TS colon lines agreed very closely with the model predictions based on breast cancer line parameters at both MTX dose levels and assay times. Depletion was nearly complete at 20 hours of exposure, as expected. Parent-line cells also agreed with model predictions at 1-μM doses of MTX. However, at 10-μM doses, 10-formyl pools in the parent line exhibited substantial experimental decline after 5 hours of exposure, while the kinetic model predicted a small increase. Possibly, the inhibition constants of the di- and triglutamated MTX species at TS are somewhat low, resulting in predictions of excessive inhibition at high MTX doses and, consequently, excessive production of the 10-formyl pool from an elevated methylene tetrahydrofolate pool. Nonetheless, the model has proven its utility in identifying a key factor controlling the proportion of direct and indirect inhibition accompanying the action of antifolates.

SIGNIFICANCE: The antifolates were long thought to exert their cell-killing action by exclusively blocking dihydrofolate reductase and preventing the maintenance of reduced folate pools necessary for purine and pyrimidine synthesis. In some cell lines, however, it was noted that these pools are not fully depleted, and that indirect inhibition at non-DHFR sites (due to the buildup of dihydrofolate behind the DHFR block) is an

important additional cause of reduced purine and pyrimidine synthesis. These non-DHFR effects are significant in that they suggest other targets for new chemotherapeutic agents. The present work shows that these secondary targets become less important with increased TS activity; conversely, if TS synthesis can be blocked by new agents, then those drugs aimed at the non-DHFR targets can be made more effective.

PROPOSED COURSE: Recent work in Dr. Allegra's laboratory has shown that TS down-regulates its own protein production rate by binding to its mRNA. Hence, a long-term goal is to identify the mRNA-TS binding region with the aim of synthesizing new peptide-containing drugs that can block TS synthesis, even in the presence of antifolates. Dr. Allegra's group has identified two of the mRNA binding sites by restriction analysis and competitive binding assays, but a third remains unknown; furthermore, no direct observation of the binding is available. Electron microscopy is therefore planned, making use of the Vacuum Generators Scanning Transmission Electron Microscope (VG-STEM) in order to visualize the protein-nucleic acid bead structure.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10337-03 BEI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Nuclear Medical Imaging: Scintigraphic Imaging System for Small Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen Markowitz, M.S.	Electrical Engineer	BEIP, NCRR
Joseph Schmitt, Ph.D.	Staff Fellow	BEIP, NCRR
Thomas Tedder	Instrument Maker	BEIP, NCRR
Burt Chidakel, B.S.	Instrument Maker	BEIP, NCRR

COOPERATING UNITS *(if any)*

NM, CC (M. Greene)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section, CCIS

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.5	0.5

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A relatively low-cost scintigraphic imaging system is being developed that is both capable of successful operation in the small-animal environment and able to carry out the full range of nuclear cardiology data acquisition and processing procedures associated with contemporary human studies. The system, with parallel hole collimation for image formation, consists of a NaI (TI) crystal coupled to a single position-sensitive photomultiplier tube; analog electronic modules to compute the position of each scintillation event; custom-designed correction circuitry for normalization of certain photomultiplier characteristics; and a Macintosh II personal computer equipped with a commercial software/hardware package designed for nuclear medicine image acquisition, processing, analysis, and display.

OBJECTIVES: Creation of a small-field-of-view scintigraphic imaging system capable of all forms of nuclear tracer imaging (i.e., planar projection imaging, R-wave or stimulus-gated imaging, time-sequence imaging, etc.).

METHODS EMPLOYED: A scintigraphic imaging system, intended to image the internal distribution of gamma ray-emitting tracers in small animals, has been designed and assembled. The spatial location of collimated photons absorbed within the crystal is calculated from signals generated by spatially dependent resistive division of the tube current, collected on crossed X and Y anode wires. Initial tests on this crystal/tube combination indicate a spatial resolution near 2 millimeters. Custom-designed nuclear instrumentation modules (NIMs) were required to correct spatial variations in energy resolution and position linearity. Pulse-height analysis and gross position computation were carried out with commercially available electronic NIMs. The corrected position inputs were then input to the Macintosh II acquistion system. An animal rotation device was added to the system to convert the system to a single photon-emission computerized tomagraphy (SPECT) imaging system for small animals.

SIGNIFICANCE: Successful completion of this project will result in a very flexible, relatively low-cost nuclear tracer imaging system, capable of yielding high-resolution images of single photon-emitting radiopharmaceuticals anywhere in the bodies of small animals such as the monkey. In particular, this system will permit novel tracer methods and new radiopharmaceuticals for studying the heart and brain, to be evaluated and screened before potential application in man. As a by-product of this work, the experience gained in the use of the Macintosh II data acquisition/processing system should allow evaluation of this system's suitability for general use with human patients. Similarly, evaluation of the position-sensitive phototube/cameras should suggest other applications for these devices.

PROPOSED COURSE: Field testing for animal studies of the planar single-camera system was performed, and modifications were made to the system. System design will be initiated for a larger-field-of-view single-camera system. A dual-camera positron emission tomagraphy (PET) system will be initiated, with additional design of custom-constructed NIMs.

PUBLICATIONS: Green MV, Markowitz A, Tedder TE, Andrich A, Neumann RD, Owens ES. SPECT imaging in small animals. In: Proceedings of the thirty-ninth annual meeting of the Society of Nuclear Medicine. Society of Nuclear Medicine, 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10339-03 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Support of Diagnostic Radiology Research Program

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ronald L. Levin, Sc.D. Biomedical Engineer BEIP, NCRR

COOPERATING UNITS *(if any)*
DRRP, OIR, OD (J. Frank)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The NIH has been requested by Congress to expand its research to include aspects of radiology and imaging. To this end, a Diagnostic Radiology Research Program has been established. This program is designed to give training in research methodology and technique to radiologists and others from the American radiological community. Part of that training shall include performing research with NIH staff scientists and clinicians on the latest techniques for whole-body magnetic resonance instrumentation, and for the visualization and analysis of multimodality medical images in multiple dimensions. Additional NMR instrumentation and image processing hardware and software are therefore being acquired. Such instrumentation will be located in a structure being constructed adjacent to the current *In Vivo* NMR Center, with easy access to its facilities, as well as to those of the Clinical Center. The BEIP is providing logistical and scientific expertise in support of the program.

OBJECTIVES:

(1) To facilitate magnetic resonance research in the fields of imaging, spectroscopy, angiography, perfusion, and diffusion.

(2) To facilitate the exchange of medically related images by permitting images from multiple sources to be accessed via a single archive and in a single file format, and to facilitate the manipulation and comparison of medically related images through the use of a specially designed biomedical workstation.

PROPOSED COURSE:

(1) A Signa 1.5-T whole-body magnetic resonance unit, to be used by this program, has been installed in the *In Vivo* NMR Research Center and has recently successfully completed acceptance testing.

(2) A multidimensional radiological image processing system (MRIPS), the primary use of which will be the visualization and analysis of multimodality medical images, is being deployed. The system consists of a combination of computer software and hardware suitable for the development of new image visualization and image analysis tools, as well as for the use of existing image processing software packages.

The MRIPS will consist of the following hardware and software: (1) a network of image visualization and image analysis workstations, supported by three centrally located data servers; (2) the UNIX operating system; (3) an X11 Windows graphic user interface; (4) software tools for the development of new image visualization and image analysis programs; (5) an image-analysis application; and (6) network and system software compatible with existing NIH computer networks and image processing programs.

The MRIPS will be used by trainees associated with the DRRP and by other NIH scientists for the analysis of medical images obtained by computerized tomography (CT), magnetic resonance imaging (MRI), magnetic resonance spectroscopy (MRS), positron emission tomography (PET), etc. Many of the studies will involve determination of the relationships between anatomical and physiological image data obtained from various body parts. Of particular importance in this regard is the need to obtain, both efficiently and accurately, spatial registration of MRIPS-collected data.

METHODS EMPLOYED: This system, the Multimodality Radiology Image Processing System (MRIPS), consists of the following components:

a. AFS (Andrew File System)-based data/computation servers, consisting of three DECstation 5000/200 CPUs with over 63.5 Gbyte of magnetic disk space; one SUN Sparcstation 2 AFS/NFS gateway; one DECstation 5000/125 AFS-client; one SUN ELC tape-backup system; associated networking and communication subsystems; and other miscellaneous peripherals.

b. Numerous AFS-based and NFS-based workstations, consisting of SUN Sparcstations, DECstation 5000s, HP Series 700 machines, and VAXstations.

c. Special dataset transfer, format conversion, and data-management routines, including Kerberos-based security mechanisms and a dataset registry.

d. The ***MEDx*** image processing software package, consisting of special medically related image processing enhancements to Sterling, Virginia-based BDS Systems' commercially available nonmedical ***Xcalibur™*** image processing package.

Currently, the NIH has licenses to use ***Xcalibur™*** on over fifty workstations, in addition to the data/computation servers. There is a large demand from members of the intramural NIH community for additional licenses to use ***MEDx*** on existing workstations, as well as the need to acquire additional workstations for those individuals/laboratories who need to do medical image processing, but currently do not have appropriate UNIX-based workstations. There is also a demand for enhancements to be made to MRIPS, in addition to long-term support and training.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10341-03 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Muscle Strength Testing System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Thomas R. Clem, Sr., BSEE Electrical Engineer EEES, BEIP, NCRR

COOPERATING UNITS *(If any)*
DIR MN, NINDS (S. Dinsmore, M. Dalakis)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects _(b) Human tissues (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

There are a variety of neurological afflictions that reduce the strength in various muscle systems in the human body. It is desirable to make an accurate quantitative measurement of the strength of various muscle groups on normal subjects, subjects who have muscle deficiencies due to disease, and subjects who are undergoing either drug therapy or physical therapy to strengthen weakened muscles. This project involves the design of a computer-based system that will allow the researcher to follow the progress of muscle strength changes over a period of time. The first application will be for patients who are suffering from Post-Polio Syndrome. The system is engineered to allow the clinician to make measurements efficiently on eighteen different muscle groups, nine on each side, in a minimum of time and with a minimum of discomfort to the test subject.

OBJECTIVES: To construct a system that will collect muscle strength data quickly and accurately in eighteen muscle groups on a test subject, and to store that data in a disk file for later analysis, along with identifying data on the subject and the test parameters.

METHODS EMPLOYED: A custom interface was designed and built to interconnect a laptop PC and a set of strain gauge force transducers. Custom programs were written to control the collection of data, plot the transducer outputs during the test, and store the data and test parameters at the end of the test.

SIGNIFICANCE: The system being designed allows the researcher to make muscle strength measurements quickly and accurately in a repeatable fashion, so that progress in strengthening (or weakening) of important muscle groups can be followed. The program being written also provides automatic data saving in a form that allows easy comparison of subsequent tests.

PROPOSED COURSE: To continue development of the program and system until it is finalized in a form that can be considered a clinical tool for measurement of muscle strength.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10342-03 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Computer-Based, Dual-Pump HPLC Driver System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
John W. Cole, MSEE Electrical Engineer BEIP, NCRR

COOPERATING UNITS *(if any)*
CE, NIDDK (R. Lippoldt)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

High-Performance Liquid Chromatography (HPLC) is a common method used to separate various molecules on the basis of their size. The solution to be analyzed is passed through a long column packed with tiny beads. Larger molecules tend to have a more difficult time passing through the column, while smaller molecules move more rapidly, leading to the desired size-separation effect. By forcing the solution through the column under high pressure, chromatographic separations are achieved much faster and with greater resolution, compared to standard chromatographic methodologies. In most typical HPLC systems, a single pump is used to drive the solution to be analyzed through the column.

Recently interest has been shown in using a dual-pump driver system. A system of this type would allow the reaction and by-products of two chemical reactants to be studied and separated as the reaction occurs and passes through the column.

OBJECTIVES: To develop a computer-based, dual-HPLC-pump driver system that allows for linear, upward-curved, and downward-curved mixing gradients. The system must also allow for complete control of the following experimental parameters: total run time, total volume of liquid pumped, gradient type (linear or curved), gradient deviation or amount of curvature, and the number of different straight-line approximations used to make up a curve.

METHODS EMPLOYED: A Metrabyte counter timer board (CTM-5) was installed in an IBM-AT compatible personal computer. The CTM-5 control software was interfaced with a custom program, written in MS-Quick Basic, to instruct the CTM-5 board to produce the required signals necessary to control the pumps.

SIGNIFICANCE: A computer-based, dual-pump HPLC system will allow various new experiments to be performed, while obtaining the benefits of computer-guided accuracy. Experiments may be repeated easily and accurately for experimental result verification.

PROPOSED COURSE: The system was completed in mid-1990 and is currently in operation. As mentioned previously, the system currently includes an IBM-AT computer. Work is now in progress to update the system to an IBM-PS2-model computer. This will involve both hardware and software reconfigurations.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10343-03 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Polynomial Spline Signal Processing Techniques

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Unser, Ph.D.	Visiting Scientist	BEIP, NCRR
Akram Aldroubi, Ph.D.	Staff Fellow	BEIP, NCRR
Murray Eden, Ph.D.	Director	BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects _(b) Human tissues X (c) Neither
_(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Conventional digital signal processing techniques consider signals that are represented by a set of uniformly spaced sample values. Although most processing algorithms are derived within a purely discrete framework, there are a variety of biomedical problems (e.g., detection of anatomical structures in images, registration) that would be better formulated by considering a signal as a continuous real-valued function defined over some domain. This study is concerned with the development of new processing techniques that represent signals by continuous polynomial spline functions. Traditionally, polynomial spline interpolation or approximation problems are approached using a matrix formulation. Our initial contribution has been to derive efficient computational algorithms using recursive digital filters. These techniques have been applied to the design of fast algorithms for image interpolation and compression. We have also derived a sampling theory for polynomial splines that generalizes Shannon's sampling theorem for band limited functions. In addition, we have defined a whole family of polynomial spline wavelet transforms that allows the expansion of continuous image functions in terms of basis functions obtained by dilatation and expansion of a single prototype. Such wavelet transforms may provide space/frequency signal decompositions that are close to optimal, according to the uncertainty principle.

OBJECTIVE: To develop new processing techniques using continuous polynomial spline representations of signals. These techniques can be useful for resolution conversions (zooming and shrinking), data compression, multiscale signal analyses, edge detection, or signal differentiation.

METHODS EMPLOYED: The whole approach is based on the premise that any polynomial spline function can be represented as a weighted sum of shifted B-spline basis functions. A signal is therefore entirely characterized by the discrete sequence of its B-spline coefficients, which can be computed efficiently by digital filtering. These algorithms have been coded in FORTRAN on a low-end biomedical image processing workstation based on a standard Apple Macintosh II.

MAJOR FINDINGS: We have derived several theoretical results characterizing the performance of polynomial spline representations as the order of the splines tends to infinity:

(1) A B-spline interpolation is asymptotically equivalent to an ideal lowpass filter (classical sinc interpolator).

(2) The optimal prefilter for B-spline approximation asymptotically tends to an ideal lowpass filter (classical anti-aliasing filter).

(3) The B-spline wavelets asymptotically tend to a modulated Gaussian (Gabor function). The B-spline wavelet transform is therefore asymptotically optimum in terms of its time-frequency localization (uncertainty principle).

Properties (1) and (2) indicate that polynomial spline approximation can be viewed as a generalization of the classical sampling approach for bandlimited signals dictated by Shannon's sampling theorem. Property (3) provides the connection with the Gabor transform, which is a classical method for time/frequency signal analysis.

SIGNIFICANCE: To provide NIH scientists with new tools for the processing of biomedical signals. To increase the efficiency of resolution conversion and display algorithms on image processing workstations.

PROPOSED COURSE: We will continue our investigation of polynomial spline pyramids and wavelet decompositions for multi-resolution signal analysis. We have also started developing a spline-based multiscale registration algorithm for the alignment of brain slices for 3-D reconstruction.

PUBLICATIONS: Unser M, Aldroubi A. Polynomial spline signal processing algorithms. In: Int Conf Acoustics, Speech, and Signal Processing. San Franscisco: IEEE, 1992;3:177-80.

Unser M, Aldroubi A, Eden M. Polynomial spline signal approximations: filter design and asymptotic equivalence with Shannon's sampling theorem. IEEE Trans Information Theory 1992;38(1):95-103.

Unser M, Aldroubi A. Polynomial splines and wavelets--a signal processing perspective. In: Chui CK, ed. Wavelets--a tutorial in theory and applications. San Diego: Academic Press, 1992:91-122.

Unser M, Aldroubi A, Eden M. On the asymptotic convergence of B-spline wavelets to Gabor functions. IEEE Trans Information Theory 1992;38(2):864-72.

Aldroubi A, Unser M, Eden M. Cardinal spline filters: stability and convergence to the ideal sinc interpolator. Signal Processing (in press).

Unser M, Aldroubi A, Eden M. The L_2 polynomial spline pyramid: a discrete multiresolution representation of continuous signals. IEEE Trans Pattern Anal Mach Intell (in press).

Unser M, Aldroubi A, Eden M. A family of polynomial spline wavelet transforms. Signal Processing (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10353-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Drug Transport in Brain

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul F. Morrison, Ph.D.	Physical Scientist	BEIP, NCRR
Peter J. Basser, Ph.D.	Mechanical Engineer	BEIP, NCRR
Robert L. Dedrick, Ph.D.	Section Chief, CHES	BEIP, NCRR

COOPERATING UNITS *(If any)*
Surgical Neurology Branch, NINDS (D. Laske, H. Bobo, D. Lieberman, K. Bankiewicz, E. Oldfield)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Theoretical transport models of microinfusion into brain tissue have been developed, treating the brain either as a rigid or deformable elastic medium into which small molecular weight or macromolecular solutions are infused at flow rates from 0.1 to 6.0 µl/min. Rigid pore theory yielded a simple expression for the concentration profile of macromolecules in grey matter for distances up to 1.5-2 cm, beyond which combined diffusion/bulk models were used because of high Peclet number. Grey matter experiments with phytohemagglutinin have confirmed a spherical spread of infusate of the correct order of magnitude. Low-compliance catheters have been designed to deliver small infusate volumes (~1µl); these catheters delivered trophic factors throughout rat grey matter, allowing transfer of material to the substantia nigra. A poroelastic deformation model has been completed which identifies key combinations of parameters required to describe deformation in homogeneous tissue. Postinfusion diffusional relaxation has been shown to be a strong determinant of pharmacodynamic effect. A 12-hr high-flow infusion was shown to increase penetration distance over low-flow technology by 0.3-1.0 cm, depending on degradation rate, when compared at a fixed pharmacodynamic metric.

RELEVANT BEIP PROJECTS: 84-125, Drug transport in brain.

OBJECTIVES: To understand the fundamental processes involved in the transport of small- and macromolecular-sized molecules in the white and grey matter of the brain. To use this information to predict the distribution of these molecules following various administration protocols, and to assess the role of transport in determining therapeutic or diagnostic outcome.

METHODS EMPLOYED: Both theoretical and experimental methods have been employed. On the theoretical side, transport models were constructed from the differential representations of Darcy flow, diffusion-bulk flow, and continuity applied to a rigid porous model of the brain. Binding, biochemical reaction, and capillary pore bulk flow and permeation were also included as appropriate sink terms. For macromolecules, simple analytic expressions were derived for concentration profiles by dropping diffusional contributions for distances up to about 1.5 cm. Numerical integration of the equations was performed for distances beyond this limit. In parallel, a Biot-type poroelastic model was also developed which includes a linear constitutive law of the deformable medium, conservation of mass and momentum of the mixture, Darcy's law, and mechanical boundary conditions. Contrast of high- and low-flow microinfusion was done by differencing the penetration distances at which each infusion fell below either a pharmacodynamic threshold or a minimal area-under-the-curve metric. Because postinfusion diffusional relaxation dominates these calculations, analytical expressions for concentrations in this time phase were developed based on distributed shell sources equivalent to the concentration profiles established just at the end of either high- or low-flowrate infusion. Experimentally, very low-compliance catheters were developed from Peek HPLC tubing and 170μ silica tubing; they could deliver test fluorescein solutions with <10% error at 0.05μl/min inlet flowrate. These were used to infuse <1μl solution into rat caudate nucleus at flowrates in the range of 0.1 μl/min. Phytohemagglutinin (PHA) was infused, followed by sectioning of the brain and development of the sections by the perioxidase method.

MAJOR FINDINGS: The most significant finding was mathematical prediction and subsequent experimental confirmation of the ability to deliver large volumes of infusate to both grey and white matter from a single cannula source. Early experiments showed that a 300-μl infusion essentially fills the entire extracellular volume of white matter in the cat brain. PHA experiments in rat brain also indicate that microinfusion can easily dose grey matter, as well; the spherical concentration profiles obtained indicated that grey matter, closely approximates a homogeneous transport medium and is thus appropriately described by our isotropic model. Pharmacodynamic comparisons of low-flow (sufficiently low to be associated with pure diffusional transport in the tissue) and high-flow microinfusion showed that the high-flow case leads to a 0.3-cm to 1.0-cm deeper radial penetration into tissue after a 12-

hour infusion, depending on degradation rates ranging from zero to 1.1E-6/sec. The same increased penetration was found whether the pharmcodynamic metric was a threshold concentration or an area-under-the-curve measure. A deformation model for anisotropic tissue has also yielded an expression relating the catheter tip infusion pressure and hydraulic conductivity tensor components, thus providing the theoretical basis for extraction of anisotropic transport parameters from experimental infusion data.

SIGNIFICANCE: Delivery of macromolecules to large volumes of brain tissue suggests that high-flow microinfusion can find application in cancer therapy, enzyme replacement therapy, and gene therapy. Competing osmotic minipump and dissolving pellet technologies are hampered by their inability to dose large volumes of tissue unless many such devices are employed simultaneously. Microinfusion offers greater control than other technologies: flowrates can be altered rapidly, unlike dissolution rates from implants; surgical intervention can be minimized; in white matter, the normal interstitial fluid flows of about 10μ/min can be overcome by the infusion pump flow, to a limit of about 1 cm; high concentrations near the source (potentially toxic), which are needed to create a high-mass flux in low-flow or pellet technologies, can be avoided in high-flow microinfusion because of the much flatter concentration profile achieved with this technology.

PROPOSED COURSE: Current theory does not fully account for either pressure-dependent or pore volume-dependent hydraulic conductivity, nor does it account for the anisotropies characteristic of white matter and white-grey interfaces. Hence, experiments are planned with the Surgical Neurology Branch, NINDS, in which infusion into the optic nerve of guinea pigs will be used to yield estimates of hydraulic permeability. Further quantification of macromolecular spread in grey matter is also planned, particularly the effects of the reaction kinetics of growth factors on penetration distances. In the long term, the brain transport model could evolve into a finite element form capable of accounting for transport in large regions of the brain with anatomically correct regional boundaries and anisotropic flows along fiber tracts.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10354-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Kidney Tubule and Epithelial Transport Studies

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Peter M. Bungay, Ph.D. Chemical Engineer BEIP, NCRR

COOPERATING UNITS *(if any)*
Laboratory of Kidney and Electrolyte Metabolism, NHLBI (P. Harris, J.-Y. Chatton, K. Spring, C. Gibson)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This project concerns the development of mathematical models of fluorescence recovery following photobleaching of dyes employed as markers of the movement of solutes and water within epithelial cell layers and isolated perfused kidney tubule segments.

RELEVANT BEIP PROJECTS: 91-100, Photobleaching Recovery for Flow Rate Determination in Kidney Tubule Perfusion; 91-151, Transport Measurements from Photobleaching Recovery Techniques.

OBJECTIVES: Mathematical modeling support is being provided to the Transport Physiology Section, LKEM, NHLBI, on projects directed at understanding mechanisms of solute and water transport across epithelia, the kidney tubule epithelium in particular.

METHODS EMPLOYED: The principal activity during this reporting period related to the development of a technique for determining the diffusivity of solutes within the lateral intercellular spaces (LIS) of epithelial cells. Epithelial monolayers were grown *in vitro* on solid supports from a culture of canine kidney epithelial cells (MDCK). The test solute was an acetyl ester derivative of the pH-sensitive fluorescent anionic dye, BCECF. When a solution of this solute bathes the apical surface of the monolayer, the cells take up the solute, convert it to parent compound and secrete BCECF into the LIS. The ability of BCECF to fluoresce can be irreversibly destroyed by high intensity light of an appropriate wavelength. Laser light of 488 nm was concentrated into a beam of rectangular cross-section (16 mm across the narrow dimension) by means of a cylindrical lens electromechanically introduced into the light path. After bleaching a selected segment of LIS for an interval of controlled duration (200-500 ms), the lens was rapidly moved out of the light path, and the light intensity was lowered 250-fold by means of an acousto-optic modulator. The wavelength of the illuminating light was also switched to 458 nm, and the subsequent fluorescence emission was videotaped for an interval of up to 2 sec. During the latter interval, fluorescence from the bleached LIS segment increased because of diffusional movement of unbleached dye along the LIS in response to the concentration gradients created by bleaching. The variation in position and time of the concentration of unbleached dye was simulated mathematically by a one-dimensional, unsteady-diffusion model with linear first-order bleaching kinetics. Marquardt-Levenberg regression of the model to the fluorescence recovery data was employed to obtain values for the diffusivity of the dye and the first-order bleaching rate constant.

MAJOR FINDINGS: The average value obtained for BCECF diffusivity within the LIS was similar to that expected for the diffusivity in free solution. To confirm this result, the determination of free solution diffusivity was attempted by fluorescence recovery measurement after photobleaching of a solution of the dye, confined in a thin film between a microscope slide and cover slip. However, this approach was not successful for the experimental conditions employed. The individual effect of the two parameters, diffusivity and bleaching rate constant, on the recovery was so similar that separate values could not be determined. These difficulties suggest that the LIS measurements need to be reexamined.

SIGNIFICANCE: Establishing the magnitude of the intercellular space diffusivity relative to that found in free solution is important for testing theories of solute-water coupling in isotonic transepithelial fluid transport. Processes involved in solute and water transport across epithelia are key components in the function of kidney, bladder, and intestinal organs. The current efforts to develop means to quantitate these processes will enhance the usefulness of such research.

PROPOSED COURSE: Efforts will continue in modifying the procedures to obtain better discrimination of the individual parameters. It appears feasible to use a similar approach to quantify the permeability of the tight junctions between the epithelial cells. Other solutes (besides BCECF) will be employed to investigate the influence of solute size and ionic charge. Growing the epithelial monolayers on permeable supports will permit transport studies under the combined effects of diffusion and convection to mimic the *in vivo* conditions more closely.

PUBLICATIONS: Flamion B, Bungay PM, Gibson CC, Spring KR. Flow rate measurements in isolated perfused kidney tubules by fluorescence photobleaching recovery. Biophysical Journal 1991;60:1229-42.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10355-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Intratumoral PO2 Measurements with Photosensitizer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
John I. Peterson, Ph.D. Chemist BEIP, NCRR

COOPERATING UNITS *(if any)*
ROB, DCT, NCI (W. King)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.3	0.3

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The fiber-optic PO_2 sensor instrumentation, which was developed previously, was used to evaluate the effect of oxygen partial pressure on the effectiveness of photodynamic therapy.

Photodynamic therapy is being evaluated in clinical trials for several different tumors. The primary mechanism for cell-kill is believed to be the generation of singlet oxygen from *in situ* molecular oxygen. The objective of this study was to measure tumor tissue oxygen tension during PDT to ascertain whether significant hypoxia occurs; and, if so, whether tumor reoxygenation occurs after cessation of light.

OBJECTIVES: Determine the dose-response relationship of oxygen in photodynamic therapy.

METHODS EMPLOYED: The fiber-optic PO_2 sensor will be used to determine the variation in oxygen levels in tumors of mice dosed with photophrin during irradiation with high intensity red light.

SIGNIFICANCE: During photodynamic therapy, oxygen combines with the dye, which concentrates in tumors and absorbs the energy of optical radiation. The oxygen is then converted to singlet oxygen, a higher energy form, which has a destructive effect on tissue. Oxygen is consumed during the treatment, and is replenished by the blood circulation. An evaluation of the effect of oxygen levels on the timing and intensity of radiation to produce tumor destruction has not been done. Also, the radiation of the tumor tissue increases the temperature substantially, and the effect of this phenomenon is not known.

MAJOR FINDINGS: Radiation of the tumors with light caused increases in temperature of several degrees, which would cause measurement error; a system was therefore devised to maintain the tumors at constant temperature during measurements with and without radiation. Tests with the PO_2 sensor in tumors of mice dosed with photofrin did not show a detectable change of oxygen level during irradiation. Curiously, tests with the sensor in abdominal tissue, not in a tumor, showed a substantial drop in oxygen level in photofrin-dosed mice during irradiation, and no change in nondosed mice.

PROPOSED COURSE: This project has been discontinued. Dr. King was doing it as a sabbatical project during the summer of 1991, while he was on leave from Bucknell University.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10357-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Electrode Heating During Magnetic Stimulation

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Bradley Roth, Ph.D. Staff Fellow BEIP, NCRR

COOPERATING UNITS *(if any)*
Medical Neurology Branch, NINDS (A. Pascual-Leone, M. Hallett)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.15	0.15	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rapid-rate magnetic stimulators can heat electrodes, causing a safety hazard. We plan to characterize the electrode heating according to electrode size, shape, and material. Our goal is to set safety standards for magnetic stimulation, and to find ways to reduce electrode heating.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To characterize the heating of electrodes during magnetic stimulation, and to find ways to reduce this heating.

SIGNIFICANCE: Magnetic stimulation is a new, noninvasive, and painless technique employed in the study of the central nervous system. Recently, magnetic stimulators have been developed that can deliver trains of stimuli at a high frequency. When rapid-rate magnetic stimulation occurs in the presence of metal (e.g., EEG electrodes), the metal can became hot enough to cause burns. This safety hazard could limit the ability of neurologists to deliver trains of magnetic stimuli and to monitor the EEG response simultaneously.

MAJOR FINDINGS: Trains of magnetic stimuli can raise the temperature of typical silver EEG electrodes significantly, perhaps by as much as 50 degrees Celsius. The amount of heating is lessened when electrodes made from metals having a lower conductivity are used. Furthermore, cuts in the electrode can disrupt the eddy current path and reduce heating.

PROPOSED COURSE: Electrode heating will first be studied experimentally, using a rapid-rate magnetic stimulator; then mathematical models will be developed to interpret the experimental data. Finally, safety standards will be set to prevent the occurrence of burns during magnetic stimulation.

PUBLICATIONS: Roth B, Pascual-Leone A, Cohen L, Hallett M. The heating of metal electrodes during rapid-rate magnetic stimulation: a possible safety hazard. Electroenceph Clin Neurophysiol 1992;85:116-23.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER

Z01 RR 10358-02 BEI

PERIOD COVERED

October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*

Heat Capacity Effects in Lipids During Unilamellar/Multilamellar Phase Changes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Courtney P. Mudd, Ph.D.	Biomedical Engineer	ACES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electronics Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*

LPB, NIAMS (N. Gershfeld); IR CH, NHLBI (R. Berger)

LAB/BRANCH

Biomedical Engineering and Instrumentation Program

SECTION

Applied Clinical Engineering Section

INSTITUTE AND LOCATION

NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Recent studies of the properties of phospholipid dispersions in water indicate that a higher-order phase transition occurs involving a spontaneous transformation from a unilamellar liquid-crystal state to a suspension of large, unilamellar vesicles upon increasing the ambient temperature; it has been suggested that the unilamellar vesicles that form are a critical state. The thermodynamic properties of this transformation have been inferred primarily from the properties of air-water surface films in equilibrium with the dispersed phospholipid phase. A more direct, and conceptually simpler, analysis of the thermodynamic properties of this higher-order transition may be attained by measurements of the temperature dependence of the heat capacity of the lipid dispersions. Since transformations of this type are believed to be intimately involved in the assembly of cell membranes, we have developed an extremely sensitive differential heat conduction calorimeter for measuring heat capacities of aqueous membrane lipid dispersions. (It should be noted that attempts to measure this transformation in commercial calorimeters have not been successful.) This instrument has certain obvious advantages over the commercial differential scanning calorimeters, notably baseline repeatability and resolution. In this transformation, to measure the heat capacity change sensibly requires a calorimeter with a sensitivity of 0.0001 cal/deg-g. We estimated the sensitivity for the energetics of unilamellar vesicle formation from multilamellar liquid crystals using the intrabilayer cohesive energy of approximately 0.01 ergs/cm^2. Assuming a one-degree temperature interval for the transition, this translates into a change of heat capacity on the order of 0.001 cal/deg-g. To assure reasonable precision, we required an instrument with a sensitivity of at least one percent of the calculated transition energy. Moreover, since only mg quantities of membrane lipids are available, we are restricted to small sample sizes (1 cc).

OBJECTIVE: To use a newly designed differential (two-cell) batch-type heat-conduction microcalorimeter to step through a set of temperatures including the critical temperature, T*, and to measure the heat capacity of the lipid sample. If we introduce small, short heat bursts into the cell with the lipid solution, the resulting pulse response will depend upon the thermal properties (thermal conductivity and heat capacity) of the lipid. We used R-C modelling techniques to show that with this particular calorimeter design, when the heat burst is applied for 20 to 30 sec, the pulse response after 40 sec is independent of the thermal conductivity. Since the peak of the differential response occurs at 43 sec, we can use that value as a measure of only the heat capacity of the sample. This feature eliminates the need to make two separate measurements (thermal conductivity and heat capacity). A custom-designed, 80286-based, digital temperature controller will be interfaced to a 80286-type microcomputer that now handles all data acquisition, analysis, and storage.

SIGNIFICANCE: Equilibrium surface film studies of DMPC (lipid) dispersions have shown that at the critical bilayer temperature, T*, a unilamellar state is formed from liposomes. It is believed that there also should also be a heat capacity change at this critical temperature. Calorimetry could provide a direct measurement of the work of bilayer adhesion, as well as verification of the value of the critical temperature.

PROPOSED COURSE: Since the calorimeter was not designed to measure heat capacity, two separate measurements are required to calibrate the instrument. In the first, water is placed in both the reference and sample cells, and the difference in output voltage is measured over a range of temperatures that includes T*. This determines the instrument's baseline (referenced to water) over the temperature range. Next, the same measurement is performed with a known aqueous KCL solution replacing the water in the sample cell. Since the heat capacities of water and KCL are known as a function of temperature, the difference between the water-vs.-water voltages and the KCL-vs.-water voltages is a direct measure of the calorimeter sensitivity to heat capacity changes. Finally, lipid vs. water is run over the same range, and the voltage differences converted to heat capacity changes (referenced to water) as a function of temperature.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10359-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Flash Photolysis Apparatus

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Allen Markowitz, M.S.	Electrical Engineer	BEIP, NCRR
Burt Chidakel, B.S.	Instrument Maker	BEIP, NCRR

COOPERATING UNITS *(if any)*
LMC, DCE, NCI (F. Friedman, R. Robinson, Y. Omata, A. Koley)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section, CCIS

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.3	0.2

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This instrument is used to measure the kinetics of CO binding to cytochromes P450 in liver microsomes from rats treated with various drugs and carcinogens. When CO is added directly to rat liver microsomes, the absorbance change at 450 nm occurs too rapidly to follow on a standard spectrophotometer. In order to observe this rapid reaction, a continuous dye-laser flash photolysis apparatus was constructed to monitor the kinetics of the absorbance change.

OBJECTIVES: The objective was the kinetic analysis of CO binding to liver microsomal P450. Computer modeling was used to characterize the kinetics and equilibria of P450-ligand interactions. For microsomes obtained from rats treated with different agents, two kinetically distinguishable types of P450 were observed. The addition of various substrates or antibodies to the microsomes altered the kinetics. The dye-laser flash photolysis apparatus was designed to measure the kinetic reactions.

METHODS EMPLOYED: The sample is pulsed with a laser flash at 530 nm to disrupt the photolabile bond between CO and the heme iron of the P450. Transmitted light at 450 nm is detected by the photomultiplier. Several optical filters and lenses both select wavelength and focus the light. The photomultiplier voltage output is amplified, filtered, input into an analog-to-digital converter, and stored on the IBM AT. The amplifier with offset was designed to limit A/D quantification noise. The hardware filter was used to limit aliasing effects.

Data from repetitive experiments were averaged to improve the signal-to-noise ratio (S/N). Further S/N enhancement was obtained by a moving point average and fast Fourier transform techniques. Curve fitting of the data was accomplished by a least squares exponential fit.

MAJOR FINDINGS: The binding of carbon monoxide to P450, with its characteristic absorbance change at 450 nm, was used as a probe for active site conformation. Such experiments have provided detailed information on the protein structure and dynamics in the heme region for several hemeproteins, but they have been less extensively applied to the P450 system.

SIGNIFICANCE: In conjunction with separately derived equilibrium binding data, the kinetic data will help to provide a more comprehensive view of the P450-substrate-CO system. From the perspective of P450 dynamics, the kinetics of CO recombination after flash photolysis is a powerful probe for the accessibility of small molecules to the heme in the protein interior. The parameters obtained by this approach include the rates at which CO diffuses from bulk solvent into the protein interior. They also yield the relative amplitudes of kinetically distinguishable pathways toward the final P450-CO complex. These parameters reflect both the conformational structure and the dynamics of the P450.

PROPOSED COURSE: Data testing is presently being performed on rat liver microsomes. Calibration procedures are being set to insure reproducibility of the data over time.

PUBLICATIONS: Markowitz A, Robinson RC, Omata Y, Friedman FK. A flash photolysis instrument with digital smoothing of data using a fast Fourier transform. Analytic Instrumentation 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10360-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Motility of Tumor Cell Metastases

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cheng Dong, Ph.D.	Staff Fellow	MES, BEIP, NCRR
Richard Chadwick, Ph.D.	Head, Theor. Biomech. Group	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
LP, NCI (L. Liotta, S. Aznavoorian)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews

X _(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Invasion and metastatic spread of malignant neoplasms are associated with active locomotion of tumor cells. This locomotion occurs at the site of the primary growth, as well as at the entrance to and the egress from blood vessels. Some factors derived from host tissues have been shown to stimulate the intrinsic motility of tumor cells in *in vitro* studies. These factors are believed to influence both the extent and the direction of tumor cell movement *in vivo* to specific target organs. Although much experimental research has been done to observe this phenomenon, the detailed molecular kinetics and biomechanical functions of the generation of cell locomotion have not been well understood (e.g., initiation and regulation of pseudopod formation under certain chemoattractants). To gain such an understanding, we are using micropipette assays to characterize individual cell behavior, from which a dynamic process of pseudopod growth can be monitored under the light microscope. The information obtained can be used for developing mathematical models through a biophysical approach, based on both molecular and continuum mechanics theories, in order to understand the mechanism of this metastatic spread.

OBJECTIVES: To use combined experimental and theoretical work to establish the biophysical processes of chemotactic responses of tumor cells more definitely.

SIGNIFICANCE: The ability of cells to perform active movements is vital to their functions in physiological and pathological processes. In tumor metastases, the cell projects pseudopods in a chemotactic gradient and migrates across tissues. Both this chemoattractant-stimulated motility and the formation of pseudopodia occur on a wide variety of adhesive substrata, suggesting that certain intrinsic motility events are independent of the attachment mechanism. The questions are then focused on our understanding of this active cell motility.

MAJOR FINDINGS: The human melanoma cell line, A2058, was used for our investigation. Chemotactic response of A2058 cells to type IV collagen (one of the extracellular matrix components) shows that the motility of the tumor cell is the principal requirement for metastasis. The first step of tumor spread occurs when a tumor cell forms a pseudopod toward a chemotactic source. Using micropipette technique, we filled a 5- to 6-mm-diameter pipette with type IV collagen and placed it near a cell. We have observed that under controlled (i.e., drug treatment-free) conditions, the pseudopod grows continuously into the pipette. The time course of the pseudopod growth is about two hours. If the cell is pretreated with pertussis toxin (PT), pseudopod growth is decreased significantly, suggesting that a signal transduction through the PT-sensitive G-protein pathway is involved. Blocking this signal could be important to the inhibition of tumor cell motility, which leads to cancer metastasis.

PROPOSED COURSE: To develop a new *in vitro* assay using micropipette technique in order to observe a dynamic growth of the pseudopod when a tumor cell is activated. Developing a model of the protrusion of pseudopodia in tumor cell metastasis is the next step, based on an assumption that actin filaments elongate due to actin polymerization at the tip of the pseudopod. The driving force of extension is hypothesized as being provided by the actin polymerization and osmotic swelling. The theoretical prediction of the time course of pseudopod growth will be validated by our experimental observation from the micropipette test.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10361-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Rheology of Sickle Erythrocytes

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Cheng Dong, Ph.D.	Staff Fellow	MES, BEIP, NCRR
Richard Chadwick, Ph.D.	Head, Theor. Biomech. Group	OD, BEIP, NCRR

COOPERATING UNITS *(if any)*
LCB, NIDDK (A. Schechter)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.2	1.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects X _(b) Human tissues (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The rheological properties of normal erythrocytes appear to be determined largely by those of the red cell membrane. In sickle cell disease, the intracellular polymerization of sickle hemoglobin upon deoxygenation leads to a marked increase in intracellular viscosity and elastic stiffness, and also has indirect effects on the cell membrane. In order to estimate the components of abnormal cell rheology due to the polymerization process (and that due to the membrane abnormalities), we have developed a mathematical model of whole cell deformability in narrow vessels. This model uses hydrodynamic lubrication theory to describe the pulsatile flow in the gap between a cell and the vessel wall. We use published values of normal and sickle cell membrane elastic modulus and of sickle hemoglobin viscous and elastic moduli as a function of oxygen saturation to estimate the cell deformation and relative hydrodynamic resistance as a function of oxygen saturation for sickle erythrocytes. The model should be useful for reconciling the vast and disparate sets of data available on the abnormal properties of sickle cell hemoglobin and sickle erythrocyte membranes--the two main factors that lead to pathology in patients with this disease.

OBJECTIVES: To increase our understanding of the relative contributions of the red cell membrane and the internal sickle hemoglobin solution to the viscous and elastic components of total cell deformability during passage through the microcirculation, particularly at different oxygen saturation levels.

SIGNIFICANCE: The deformability of erythrocytes is a critical determinant of flow in the microcirculation. Abnormalities of these properties are thought to be the principal cause of the pathophysiology of sickle cell disease and other hemoglobinopathies. Extensive clinical studies of the effects of the abnormal sickle erythrocytes in patients have been reported. A central question in sickle cell rheology remains, however: to ascertain the relative importance of the sickle hemoglobin and cell membrane to the overall properties of sickle cells in the microcirculation.

MAJOR FINDINGS: The model indicates that a cell becomes less and less deformable when oxygen saturation drops below 40% to 50%, or when polymer fraction rises above 0.4, if the membrane rigidity has a value representative of a normal cell. The flow resistance of the cells increases significantly when oxygen saturation decreases to this critical level. Cell membrane elasticity certainly becomes a major determinant of the overall cell deformability when sickled hemoglobin solution is fully oxygenated. Several factors can contribute to altering the critical value of polymer fraction (or oxygen saturation). For example, increasing the oscillatory frequency of the blood flow tends to shift the critical value of polymer fraction downward. A membrane rigidity dependence on oxygen saturation can significantly alter this value, as well; in fact, the concept of a critical polymer fraction no longer applies. In other words, small amounts of polymer can significantly affect whole cell deformability in this case.

PROPOSED COURSE: The model will be extended to take into account the situation in which a cell passes through a very narrow capillary (i.e., the tube dimension is smaller than that of the undeformed particle). We hope that the model will provide a framework for analyzing the components of sickle cell rheology as more experimental data become available. A filtration assay of sickle cell trait (AS) suspension using nickel mesh has been designed. Both experimental and theoretical investigations are making progress.

PUBLICATIONS: Dong C, Chadwick RS, Schechter AN. Influence of sickle hemoglobin polymerization and membrane properties on deformability of sickle erythrocytes in the microcirculation. Biophysical Journal (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10362-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Binding Forces in Receptor-Mediated Cell Adhesion

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Alec Eidsath, Ph.D. Staff Fellow MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LTCB, NCI (C. Saxinger)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

X _(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Since it is known that the *tat* protein of the HIV will enter the cell *in vitro* and stimulate the production of HIV products, there is almost certainly a receptor site for it on the cell membrane. Segments of this 86 amino acid protein are being synthesized to determine which segment is responsible for binding to the receptor. This phenomenon will be evaluated by quantitatively measuring the binding force between the various segments and several types of human cells grown in tissue culture. We hope to use a flow cell and image analysis to determine the average shear force needed to detach the cells. The cells will then be attached to the bottom of a 96-well plate, which will be moved automatically from well to well under computer control.

OBJECTIVES: To rank the binding energies of various *tat* fragments by determining the force necessary to remove bound cells.

METHODS EMPLOYED: We have chosen two methods of applying force to a cell: centrifugal force and shear force. For the experiments using centrifugal force, we will bind cells to polypeptides bound to the bottoms of 96-well plates, and then spin the cells in a medium of a higher specific gravity. Cells that are pulled off are then aspirated, and the number of bound cells is measured using a fluorescence reader. This method is limited by the force that can be put onto the microtitre plates themselves. Fragments that are more tightly bound can then be removed by centrifugal force, and will be analyzed using a flow system, which can generate strong shear forces. Using the flow system together with a microscope makes it possible to visualize directly how the cells deform and eventually separate from the wall. Since the synthesis of the fragments takes place in a 96-well plate, it is very convenient to base the design of the flow cell on the geometry of the plate. We have decided to use a stagnation point flow (a jet of liquid impinging on the surface containing the cells). The shear stress is inversely proportional to the radius, so one would expect to see a clear ring near the center of the well where the flow was stronger than the binding energy, and then a resumption of attached cells at a radius where the binding force just overcomes the shear force. An image-analysis system will be set up to determine this critical radius automatically (the cells will be loaded with a fluorescent dye, and the image will be taken in an epifluorescence setup). The microscope and flow system must also be designed to fit in a flow hood inside a biohazard room, due to the potential dangers of the virus being studied.

SIGNIFICANCE: We think that the binding studies are an excellent tool to complement fundamental molecular biology studies. The direct aim of the project is to determine which amino acids are involved in the binding of the cell to the surface receptor. We intend to compare these results to the three-dimensional configurations produced using molecular dynamics programs. This research will also enable us to better understand the basic immunology of the virus.

PROPOSED COURSE: The centrifuge work has been completed. Preliminary studies indicate that the radial flow will generate sufficient wall shear stress to remove the bound cells, so we have gone ahead with the design of an automated instrument that will increase the throughput of data. This design is complete, and is now in the fabrication stage.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10363-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Current Dipole Localization Using EEG Data Model

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bradley Roth, Ph.D.	Staff Fellow	BEIP, NCRR
David Kovar, B.S.	Summer Student	BEIP, NCRR

COOPERATING UNITS *(if any)*
Medical Neurology Branch, NINDS (M. Hallett, S. Sato, L. Cohen, M. Balish, A. Gorbach)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A computer program will be developed that can determine the position, orientation, and strength of a current dipole in the brain from electroencephalographic or magnetoencephalographic data. The electrical properties of the head will be modeled using a three-layer model: brain, skull, and scalp. A realistic head shape will be used. The program will be used to localize the focus of epileptic discharges and to analyze evoked potentials. Comparisons will be made between the realistically shaped head model and the three-sphere model.

OBJECTIVES: To localize an equivalent dipole source in the brain, using electroencephalographic (EEG) or magnetoencephalographic (MEG) data.

SIGNIFICANCE: The localization of the source of the EEG and MEG is an old problem that has been studied extensively. Until recently, the primary model used to analyze EEG and MEG data was the three-sphere model. It has become clear recently that there are significant errors introduced by modeling the head as a sphere. Thus, by using a realistic head geometry, more accurate localizations can be obtained. This can improve our understanding of human cortical organization, and enable surgeons to have more precise knowledge of the areas of the brain responsible for epileptic seizures.

MAJOR FINDINGS: Comparisons between the three-sphere model and the realistically shaped head model indicate that average differences of 2 cm are observed between the predicted dipole position between the two models.

PROPOSED COURSE: The first goal will be to verify the model by comparing it to the three-sphere model. Then the sensitivity of the model to various parameters, such as skull conductivity, will be determined. Random noise will be added to the simulations to determine the influence of measurement noise on the results. Finally, the model will be used to analyze experimental data.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10365-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Study of Polarized Light Propagation in Scattering Media

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joseph M. Schmitt, Ph.D.	Staff Fellow	BEIP, NCRR
Robert F. Bonner, Ph.D.	Biophysicist	BEIP, NCRR

COOPERATING UNITS *(if any)*
PSL, DCRT (A. Gandjbakhche)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

This study is concerned with the theory and application of polarized light transport in scattering media. We have identified the conditions under which photons maintain their polarization after being scattered multiple times. Our results suggest several novel applications, including the measurement of the optical activity of chiral substances, such as glucose, in thin tissue specimens. Several novel experimental solutions to the problem of measuring small polarization fractions and optical rotations have also been developed in the course of this work.

OBJECTIVES: To address fundamental questions concerning the propagation of polarized light in scattering media, and to investigate new techniques for quantifying regional concentrations of optically active substances in tissues.

METHODS EMPLOYED: An optical system employing a photoelastic modulator was constructed to measure extremely small polarized light intensities in a background of scattered, depolarized light. To gain a better understanding of the randomization of polarization by multiple scattering, measurements were made on light-scattering samples composed of polystyrene beads suspended in water, and compared with the results of Monte Carlo simulations. The effect of the size of the scattering particles on the optical pathlength over which depolarization occurs was also examined.

We added varying concentrations of dextrose to the polystyrene suspensions to test if changes in the concentration of an optically active substance dissolved in a suspension of scattering particles can be measured despite the randomization of polarization that occurs as a result of multiple scattering.

MAJOR FINDINGS: With the present experimental apparatus, we have measured polarization fractions as small as 1×10^{-3} in samples with optical thicknesses comparable to about 5 mm of skin tissue. The near-ballistic nature of the polarized-photon paths has been demonstrated by experiments in which absorbing barriers were detected with good contrast in optically dense suspensions of submicron-sized polystyrene spheres.

Because only a small fraction of the initially polarized light remains polarized after propagating through an optically dense medium, measurement of small optical rotations is difficult. Even using our sensitive apparatus, the lowest concentration that we have succeeded in measuring through a tissue phantom having an optical density comparable to 5 mm of tissue was 20 times greater than that normally found in blood.

SIGNIFICANCE: Little is known about the propagation of polarized light in optically dense substances. Results of this study have provided new insights into the effects of multiple scattering on polarized light propagation. In the course of this work, several ideas for potential uses of polarized light measurement have arisen, including the following: (1) a means of imaging structures imbedded in multiply scattering samples; (2) a simplified method for determining the asymmetry parameter of an optical scatterer; and (3) techniques for measuring the concentration of optically active substances in a volume of scatterers.

PROPOSED COURSE: We plan to continue the development of sensitive optical methods for detecting extremely small optical rotations. In particular, reflection-mode methods that promise to

reduce detection limits will be investigated. Semiconductor lasers that exhibit enhanced polarization stability will be employed to reduce drift, which has severely limited the precision of previous measurements. These improvements should enable us to answer several important questions regarding the propagation of polarized light in optically active scattering media.

PUBLICATIONS: Schmitt JM, Gandjbakhche AH, Bonner RF. Use of polarized light to discriminate short-path photons in a multiply scattering medium. Applied Optics (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10366-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Noninvasive Measurement of Arterial Blood Hematocrit

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joseph M. Schmitt, Ph.D.	Senior Staff Fellow	BEIP, NCRR
Guanxiong Zhou	Visiting Scientist	BEIP, NCRR

COOPERATING UNITS *(If any)*
None

/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
  _(a1) Minors
  (a2) Interviews

_(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We are developing a photoplethysmographic technique for assessing arterial blood hematocrit noninvasively. The intensity of light transmitted through a blood-perfused tissue is measured at two near-infrared wavelengths. Based on the results of both theoretical and experimental studies, the measured intensities are calibrated in terms of blood hematocrit.

OBJECTIVES: To investigate a method for measuring arterial blood hematocrit by diffuse-transmittance near-infrared spectrophotometry of the skin. Used in conjunction with pulse oximetry, this method may enable the oxygen saturation and concentration of hemoglobin to be monitored simultaneously in critically ill patients.

METHODS EMPLOYED: Several isosbestic regions of oxy/deoxy-hemoglobin exist in the near-infrared spectrum. The ratio of the pulsatile (AC) and average (DC) light intensities transmitted through the finger or earlobe at wavelengths within two of these isosbestic regions is proportional to the concentration of hemoglobin in the blood. Feasibility studies using tissue phantoms have been undertaken to determine if arterial hematocrit can be assessed transcutaneously by measuring this ratio. Several solid-state sensors, employing InGaAlAs light-emitting diodes and an InGaAs photodiode, have been constructed for use in these studies.

To understand the effects of potentially interfering variables on the relationship between blood hematocrit and the measured AC-DC ratios, we have employed computer models of photon diffusion in tissues.

MAJOR FINDINGS: Our experimental results show that the diffuse transmittances of whole blood measured at 805 nm and 1300 nm can be calibrated in terms of the total concentration of hemoglobin, independent of its state of oxygenation. Although influenced by physiological variables such as total blood volume and tissue water content, transcutaneous measurement of hematocrit using ratiometric photoplethysmography appears feasible. Preliminary results obtained from measurements on live human skin suggest that calibration could be performed empirically, in a manner similar to that now employed to calibrate pulse oximeters.

SIGNIFICANCE: Monitoring both arterial hemoglobin concentration and oxygen saturation during and after surgery would enable tighter control of a patient's cardiopulmonary status. Noninvasive hematocrit monitoring may enable detection of occult bleeding during surgery, better control of anemia in dialysis and sickle-cell patients, and periodic screening for anemia in developing countries. Hazards associated with blood sampling in these applications would be eliminated.

PROPOSED COURSE: After receiving inquiries from several companies, we filed a patent application to facilitate licensing of the hematocrit-monitoring methods. We anticipate further development of the method by manufacturers of pulse oximeters interested in adding new measurement capabilities to instruments now on the market. To accomplish this aim, they must first conduct studies using prototype instruments to test the viability of the methods in a clinical setting.

PUBLICATIONS: Schmitt JM, Zhou GX, Miller J. Measurement of blood hematocrit by dual-wavelength near-IR photoplethysmography. Proc. SPIE 1992;1641:150-61.

Schmitt JM. US Patent Appl 07/822,018: Optical method for monitoring arterial blood hematocrit.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10367-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Imaging of Biological Tissues Using High-Frequency Intensity-Modulated Light

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Joseph M. Schmitt, Ph.D. Staff Fellow BEIP, NCRR

COOPERATING UNITS *(if any)*
IR CE, NHLBI (A. Knüttel, R. Balaban); IR LCB, NHLBI (J. Knutson)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this study, we are exploring imaging applications of high-frequency diffusing-wave spectrophotometry. A turbid medium is illuminated at one or more points on its surface with modulated light from a mode-locked laser. By measuring the phase shift and modulation depth of light re-emitted from the surface of the medium, we attempt to locate absorbers embedded in the medium. Preliminary results show that by taking advantage of the coherent properties of the propagation of diffusing waves in a homogeneous medium, detection of absorbers located deep (one or two centimeters) below the surface can be accomplished.

OBJECTIVES: To develop a method for imaging the concentration of intrinsic absorbers or contrast agents imbedded in living tissue. New techniques will be investigated that are based on diffusing-wave spectrometry using high-frequency (100 MHz to several GHz) sources.

METHODS EMPLOYED: Toward the goal of imaging absorbers in tissues, a variety of methods are being studied for deducing the spatial distribution of photons inside tissues from time-of-flight profiles measured on the surface of the skin. We have developed a novel approach to this problem, based on the interference of high-frequency photon-density waves propagating through soft tissues. By illuminating the surface of a tissue using an array of point sources having different relative magnitudes and phases, waves generated in the scattering volume are made to interfere destructively at desired locations. In the region of destructive interference, sensitivity to changes in absorption are reduced or eliminated, whereas in nearby regions sensitivity is enhanced.

A rather elaborate apparatus, consisting of a mode-locked laser, acousto-optic beam-steering optics, and a gated phase-sensitive CCD camera, has been assembled to conduct experiments with tissue phantoms. Using this apparatus, we have carried out a wide variety of imaging studies.

To facilitate experimental design of experiments and the interpretation of experimental results, analytical and numerical (finite-difference) models have been developed. These models have also been used to quantify the resolution and contrast attainable using various imaging approaches.

MAJOR FINDINGS: Phenomena associated with the interference of photon-diffusion waves have been studied in detail, both experimentally and theoretically. We have demonstrated that the position of an absorbing or fluorescent object located about 1 cm below the surface of a tissue phantom can be determined very precisely by using interference-based reflectance-mode imaging techniques. Resolution of two or more closely spaced objects has proven to be much more difficult, however, because of constraints imposed by the relatively long wavelengths of the photon-density waves in tissue-like media.

SIGNIFICANCE: Although light absorption by most biological tissues throughout much of the visible/near-infrared spectrum (630 to 1300) is relatively low, penetration is shallow because light is multiply scattered. Knowledge of the time histories of photons (or, equivalently, the relative phase-shifts of the photon flux as a function of time) emerging from a scattering medium can form the basis of techniques for reconstructing probable photon sample volumes. This opens the possibility of imaging the concentration of absorbers, especially hemoglobin and myoglobin, in muscle or other tissues. Because it is nonionizing, visible/near-infrared light is not expected to be harmful to living tissues, as long as energy densities are kept low enough to avoid thermal damage;

therefore, truly noninvasive determination of the concentration of substances that absorb light in this wavelength range may be achievable.

PROPOSED COURSE: We intend to expend more effort on defining the fundamental limits of resolution and contrast that are attainable using high-frequency diffusing-wave spectroscopy. A combined theoretical and experimental approach will be employed to answer these important questions. Studies will be performed on excised biological tissue specimens to explore the effects of scattering nonhomogeneity on imaging *in vivo*. We also plan to confront the numerous challenges involved with producing images of absorbers embedded in a tissue from phase and magnitude data collected from the tissue's surface.

PUBLICATIONS: Schmitt JM, Knüttel A, Knutson JR. Interference of diffusive light waves. J Opt Soc Am A (in press).

Knüttel A, Schmitt JM, Knutson JF. Spatial localization of absorbing bodies by Interfering diffusive photon-density waves. Applied Optics (in press).

Knüttel A, Schmitt JM, Knutson J. Improvement of spatial resolution in reflectance near-IR imaging by laser-beam interference. Proc SPIE 1992;1640:405-16.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10368-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biopsy Needle Locator

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Joseph Schmitt, Ph.D.	Staff Fellow	BEIP, NCRR
Guanxiong Zhou	Visiting Scientist	BEIP, NCRR

COOPERATING UNITS *(if any)*
DR, CC (T. Shawker)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The tip of a biopsy needle is often not clearly discernible using a standard ultrasonic scanner. We are attempting to devise a means of vibrating or rotating a biopsy needle to permit visualization by color Doppler-flow ultrasound during clinical procedures. Last year, we constructed a device using a modified speaker coil. The colored regions produced by the vibration were found to be unsatisfactory. This year, we developed two other types of devices for producing small mechanical disturbances to improve visualization.

PHS 6040 (Rev. 5/92)

OBJECTIVES: To design and construct a prototype device that will permit clinicians to locate the tip of the needle during biopsy procedures, using a color-Doppler ultrasound scanner.

METHODS EMPLOYED: Ultrasonic imaging systems capable of detecting blood flow in a static tissue background are now available. Flow direction and magnitude are indicated by colored regions superimposed on the gray-level image display. Taking advantage of this motion-sensing capability, we designed and constructed several types of devices to perturb a biopsy needle mechanically in order to create false flow images indicating the position of the needle. The following designs were tested:

1. A vibrator was constructed for attachment to the proximal end of a biopsy needle, using a modified solenoid (which is more powerful than a simple speaker coil). Driven by a function generator, the solenoid can create a variety of motions.

2. A tiny DC motor was attached to a biopsy needle. Using a function generator, the needle was rotated forward and backward at a low speed to simulate turbulent flow.

MAJOR FINDINGS: Although the colors produced by a moving needle are easily seen, they tend to be spread out within a wide area surrounding the needle shaft because the sample volume of the Doppler-flow detection system is large. This is an inherent limitation of the ultrasonic scanner (for a given frequency of the scanning probe) that cannot be overcome by altering the design of the needle vibrator. Nonetheless, the latest needle vibrators that we have designed may still prove useful in the clinic.

SIGNIFICANCE: The tip of a biopsy needle is not clearly discernible using a standard ultrasonic scanner. As a result, the success of the biopsy procedure depends greatly on the skills and experience of the clinician. A rapid and simple-to-use technique for visualizing the biopsy needle tip would revolutionize ultrasound-guided biopsy porcedures.

PROPOSED COURSE: Dr. Shawker intends to evaluate the utility of the needle locator while performing future biopsy procedures. It appears that the problem of poor resolution cannot be overcome unless the biopsies can be performed using higher-frequency transducers.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10369-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Molecular Modeling of Oligo-DNA

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ching-Nien Chen, Ph.D. Physical Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Molecular modeling of various conformations of oligo-DNA double- and triple-stranded segments has been performed, in order to gain insight into the possibility of residues from a third strand in the vicinity of a double helix. This technique provides a tool for studying the possibility of structural enhancement, or the blocking of it, from certain nucleic acid analogues to the formation of triple helices. This tool, in turn, aids the design of synthetic drugs.

PHS 6040 (Rev. 5/92)

PUBLICATION: Ono A, Chen C-N, Kan LS. DNA triplex formation of oligonucleotide analogs consisting of linker groups and octamer segments which have opposite sugar-phosphate backbone polarities. Biochemistry 1991;30:9914-21.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10373-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Operation of the *In Vivo* NMR Research Center

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Chrit Moonen, Ph.D.	Manager, NMR Center	BEIP/NCRR
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	BEIP/NCRR
Alan Olson, M.S.	Special Expert	BEIP/NCRR
Daryl DesPres, B.S.	Biologist	BEIP/NCRR
Denise Sobering, B.A.	Clerk/Typist	BEIP/NCRR

COOPERATING UNITS (if any)
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	1.3	1.4

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The day-to-day operations of the *In Vivo* NMR Research Center include, but are not limited to, the following tasks: scheduling of usage of magnets and computers; ordering supplies stocked by the Center for all users; maintenance and administration of the magnet and computer systems; and other activities necessary for a shared resource. In addition, we perform various tasks directed at the basic operation of the laboratory (accounting for usage and funding, submission of necessary documentation, telephone inquiries, information dissemination, etc.).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10374-02 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT (80 characters or less. Title must fit on one line between the borders.)
Functional Magnetic Resonance Imaging and Spectroscopy in Medicine and Biology

PRINCIPAL INVESTIGATOR (List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)

Chrit Moonen, Ph.D.	Manager/NMR Center	BEIP/NCRR
Guoying Liu, Ph.D.	Post-Doctoral Fellow	BEIP/NCRR
Geoffrey Sobering, Ph.D.	Senior Staff Fellow	BEIP/NCRR
Alan Olson, M.S.	Special Expert	BEIP/NCRR
Daryl DesPres, B.S.	Biologist	BEIP/NCRR

COOPERATING UNITS (if any)
(See summary below and list at top of next page.)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.7	1.3	1.4

CHECK APPROPRIATE BOX(ES)
X _(a) Human subjects _(b) Human tissues (c) Neither
_(a1) Minors
_(a2) Interviews

SUMMARY OF WORK (Use standard unreduced type. Do not exceed the space provided.)

The purpose of the *In Vivo* NMR Research Center is the advancement of *in vivo* NMR technology, its applications to animal and human physiology, and, ultimately, its application in the clinical setting. The research of the NMR Center's staff is focused on new methods and original applications of functional magnetic resonance methods. The following methods are being developed or applied: flow imaging; imaging of capillary circulation; diffusion of water and metabolites in brain and muscle; imaging of oxygen consumption; imaging and spectroscopy of aerobic and anaerobic glucose metabolism and transport; imaging of altered metabolism in the brain, such as the types of anomalies due to tumors, Alzheimer's disease, and multiple sclerosis; localized spectroscopy to follow metabolism and pharmacokinetics; F-19 spectroscopy to measure aldose reductase activity in the eye (with Robert Balaban, Ph.D., Chief/Cardiac Energetics, IR/NHLBI; Peter Kador, M.D., LMOD/NEI; K. Karino, Ph.D., NEI; and K. Mori, Ph.D., NEI); development of the use of field gradients in high resolution NMR spectroscopy (with Peter van Zijl, Ph.D., Johns Hopkins Univ.); development of multivolume localized spectroscopy; and eddy current compensation on 2-T and 4.7-T CSI instruments.

ADDITIONAL SCIENTIFIC COLLABORATORS:

Joe Gillen		NMR, Pittsburgh, Pennsylvania
Giovanni DiChiro, M.D.	Chief/Neuroimaging	DIR/NINDS
Jeffry Alger, Ph.D.	Expert/Neuroimaging	DIR/NINDS
Jeffrey Zigun, M.D.	Fellow, CBDB	MHIRP/NIMH
Daniel Weinberger, M.D.	Chief, CBDB	MHIRP/NIMH
Joseph Frank, M.D.	Acting Director	DRRP/OD
Alan McLaughlin, Ph.D.	Chief/Flow Lab	NIAAA
Robert Turner, Ph.D.	Visiting Scientist	IR/NHLBI
Denis LeBihan, M.D.	Fogarty Scientist	DR/CC

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van Zijl PCM, Ligeti L, Sinnwell T, Alger JR, Chesnick AS, Moonen CTW, McLaughlin AC. F-19 NMR measurement of localized cerebral blood flow. Magn Res Med 1990;16;489.

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van Zijl PCM, Moonen CTW. Solvent suppression strategies for *in vivo* magnetic resonance spectroscopy. NMR, Basic Principles and Progress 1991;26:67.

van Zijl PCM, Moonen CTW, Faustino P, Pekar J, Kaplan O, Cohen JS. Complete separation of intracellular and extracellular information in NMR spectra of perfused cells by diffusion-weighted spectroscopy. Proc Natl Acad Sci USA 1991;77:3228.

Vervoort J, Rietjens IMCM, Moonen CTW, von Kienlin M, DesPres D. Biotransformation of 2-fluoroaniline in rats studied by *in vivo* NMR. NMR in Biomedicine 1991;4:255.

Pekar J, Ligeti L, Ruttner Z, Lyon RC, Sinnwell TM, van Gelderen P, Fiat D, Moonen CTW, McLaughlin AC. *In vivo* measurement of cerebral oxygen consumption and blood flow using O-17 magnetic resonance imaging. Magn Reson Med 1991;21:313.

Turner R, LeBihan D, Moonen CTW, DesPres D, Frank JA. Echo-planar time course MRI of cat brain oxygenation changes. Magn Reson Med 1991;22:159.

Vervoort J, van Berkel WJH, Müller F, Moonen CTW. NMR studies on p-hydroxybenzoate hydroxylase from *Pseudomonas fluorescens* and salicylate hydroxylase from *Pseudomonas putida*. Eur J Biochem 1991;200:731.

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Fiat D, Ligeti L, Lyon RC, Ruttner Z, Pekar J, Moonen CTW, McLaughlin AC. *In vivo* O-17 NMR study of rat brain during O2-17 inhalation. Magn Reson Med 1992;24:370.

Moonen CTW, van Gelderen P, Vuister G, van Zijl PCM. Gradient-enhanced exchange spectroscopy. J Magn Reson 1992;97:419.

Ruiz-Cabello J, Vuister G, Moonen CTW, van Gelderen P, Cohen JS, van Zijl PCM. Gradient-enhanced heteronuclear correlation spectroscopy: theory and experimental aspects. J Magn Reson (in press).

McLaughlin AC, Pekar J, Ligeti L, Puttner Z, Lyon RC, Sinnwell T, van Gelderen P, Fiat D, Moonen CTW. *In vivo* measurement of cerebral blood flow and oxygen consumption using O-17 magnetic resonance imaging. In: Zackary S, ed. Imaging in alcohol research. Washington, D.C.: U.S. Government Press, 1992;273-86.

McLaughlin AC, Pekar J, Ligeti L, Moonen CTW. Oxygen-17 magnetic resonance imaging of cerebral blood flow and oxygen consumption. In: Rosen BR, LeBihan DL, eds. Magnetic resonance imaging of diffusion and perfusion (in press).

Moonen CTW, Liu G, van Gelderen P, Sobering G. A fast gradient-recalled MRI technique with increased sensitivity to dynamic susceptibility effects. Magn Res Med (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10378-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mechanisms of Angioplasty and Atherectomy of Coronary Stenoses

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Robert Bonner, Ph.D.	Physicist	BEIP, NCRR
Philippe Douek, M.D.	Guest Researcher	BEIP, NCRR

COOPERATING UNITS *(if any)*
Cardiology Branch, NHLBI (S. Epstein, E. Unger); Intratherapy, Inc.; Washington Cardiology Center; SCIMED, Inc.

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

As part of an effort to develop new, more effective forms of angioplasty, we have sought improved understanding of the nature of focal coronary stenoses and their acute and chronic responses to intervention. Through the development of the clinical use of intravascular ultrasound (IVUS), we have shown that the composition and stiffness at focal chronic stenoses is remarkably different from adjacent, angiographically normal vessel segments, whereas the total mass of atheroma is remarkably similar. The principal lesions currently treated by angioplasty are rigid due to fibrosis and calcification, which prevents compensatory dilatation observed in more compliant (though heavily diseased) segments. Accordingly, successful interventions disrupt/alter the stiff constraining elements so as to make them sufficiently and irreversibly compliant and permit vessel expansion. Successful therapy requires segmental (limited) rupture of the stiff annulus and creation of a large, compliant arc. Acoustic transients created by rapid bubble expansion are the principal cause of this disruption in laser angioplasty. Directional atherectomy creates deep focal excisions which can make a small arc (~60°) highly compliant. Rotablators remove luminal calcification, thereby reducing wall stiffness. Following expansion of "rigid" metallic stents at high pressures, compressive forces are generated by the surrounding tissues which cause significant acute and chronic recoil. Such acute and chronic compressive narrowing of treated lesions may be the major cause of restenosis. Transient, moderate (~60°C) thermal elevations associated with thermal angioplasty elicit a profound, dose-dependent, proliferative response similar to that seen with severe mechanical injury.

OBJECTIVES: Current objectives are: (1) to determine the mechanisms of success and failure of angioplasty/atherectomy with devices now used clinically; (2) to design new devices and methods of angioplasty, based on this new understanding; (3) to develop guidelines for optimal selection of available devices, based on specific lesion characteristics; and (4) to decrease restenosis rate.

METHODS EMPLOYED: Quantitative morphometry based on both clinical intravascular ultrasound measurements, and pathology of atherectomy specimen and postmortem coronaries. Development of animal models of proliferative responses to vascular injury and *in vitro* assessment of device performance. Final evaluation of the angioplasty devices will be based on quantitative intravascular ultrasound examination of clinical lesions before and after therapy, in conjunction with long-term follow-up.

MAJOR FINDINGS: (1) We have shown that the composition and stiffness at focal chronic stenoses is remarkably different from adjacent, angiographically normal vessel segments, whereas the total mass of atheroma is remarkably similar. (2) The prinicipal lesions currently treated by angioplasty have become stiff due to fibrosis and calcification, which prevents compensatory dilatation observed in more compliant (though heavily diseased) segments. (3) Successful intervention relies on disruption/alteration of stiff constraining elements so as to make them sufficiently and irreversibly compliant, thus permitting acute and chronically maintained vessel expansion. Successful angioplasty, therefore, works by segmental (limited) rupture of the stiff annulus and creation of a large compliant arc. (4) Acoustic transients created by rapid bubble expansion during pulsed-laser angioplasty similarly disrupt the stiff vessel segment. (5) Directional atherectomy can create a deep focal excision that makes a small arc (~60°) highly compliant, frequently to the point of becoming a pseudo-aneurism. (6) Rotablators remove a luminal layer of calcification, subsequently allowing relatively low pressure disruption of the remaining lesion. (7) Following expansion of "rigid" metallic stents at high pressures, compressive forces are generated by the surrounding tissues which cause significant acute and chronic recoil. (8) These findings suggest that acute and chronic compressive narrowing of treated lesions may be a major contributor to restenosis. (9) In animal models of restenosis, we have demonstrated that transient, moderate (~60°C) thermal elevations associated with thermal angioplasty elicit a profound, dose-dependent, proliferative response similar to that seen with severe mechanical injury.

SIGNIFICANCE: Development of more efficacious means of relieving atherosclerotic obstructions in human blood vessels (particularly in the coronary arteries) or reducing restenosis could have a dramatic effect on the practice of cardiology and vascular surgery. A greater understanding of the composition and structure of atherosclerotic stenoses, as determined by IVUS and their

responses to specific devices, could lead to more efficacious selection of devices and refinement of technique.

PROPOSED COURSE: Continued development of IVUS database and analysis of pre- and postintervention vessel wall morphology in patients undergoing angioplasty. Quantitative testing of models of angioplasty and vessel wall compliance changes. Testing of drugs delivered locally to sites of vascular injury in order to inhibit smooth muscle cell proliferation in the animal models developed.

PUBLICATIONS:

Douek P, Bonner RF. Fluorescence-guided pulsed dye laser-assisted angioplasty. Radiology 1992;182:897L.

Douek PC, Correa R, Neville R, Unger EF, Shou M, Banai S, Ferrans VJ, Epstein SE, Leon MB, Bonner RF. Dose-dependent smooth muscle cell proliferation induced by thermal injury with pulsed infrared lasers. Circulation 1992 (in press).

Banai S, Shou M, Correa R, Jaklitsch MT, Douek PC, Bonner RF, Epstein SE, Unger EF. Rabbit ear model of injury-induced arterial smooth muscle cell proliferation: kinetics, reproducibility, and implications. Circ Res 1991;69:748-56.

Bartorelli AL, Neville RF, Keren G, Potkin BN, Almagor Y, Bonner RF, Gessert JM, Leon MB. *In vitro* and *in vivo* intravascular ultrasound imaging. European Heart Journal 1992;13:102-8.

Keren G, Bartorelli AL, Bonner RF, Douek PC, Leon MB. Intravascular ultrasound examination of coronary stents. In: Tobis JM, Yock PG, eds. Intravascular ultrasound imaging. New York: Churchill Livingstone, 1992;219-30.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10379-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Influence of Cell Heterogeneity on Sickle Cell Rheology

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Cheng Dong, Ph.D. Staff Fellow MES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LCB, NIDDK (C. Noguchi)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects X _(b) Human tissues (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The rheological behavior of erythrocytes is an important determinant of microcirculatory blood flow. Although the crisis of sickle erythrocytes (SS cells) is highly dependent on the oxygen saturation level, little quantitative analysis has been done to determine the influence of cell heterogeneity on cellular deformability. It is therefore necessary to determine the role of sickle hemoglobin (Hb S) concentration on, and correlate SS cell rheology to, the effect of increased cellular hemoglobin concentration upon deoxygenation. Some of these concerns were quantitated in the course of this investigation, using a simple mathematical model of whole cell deformability in narrow vessels that combines previously published data of other investigators. During Hb S polymerization, the intracellular elasticity (or static rigidity) and viscosity (or dynamic rigidity) are functions of both oxygen saturation and Hb S concentration. The transition from cell membrane to internal polymer dominance of deformability depends on critical oxygen levels, with widely distributed Hb S concentration, at which sickling takes place. Thus, the variablility of intracellular Hb S concentration is a characteristic feature of SS erythtocytes, and an important factor for the severity of the disease.

OBJECTIVES: To study the effects of nonideality and cell hemoglobin concentration on the polymerization of Hb S in cells, and to establish some understanding of the role of cell heterogeneity in sickle cell deformability.

SIGNIFICANCE: An increasing body of experimental evidence demonstrates that intracellular hemoglobin concentration and composition are primary determinants of pathophysiology in sickle cell disease. Polymer is formed predominantly in the dense cells at very high oxygen saturation values. Heterogeneity in intracellular hemoglobin concentration causes the critical oxygen saturation necessary in the formation of polymer to shift.

MAJOR FINDINGS: The results showed that heterogeneity in increased intracellular hemoglobin concentration causes an upward shift in the critical oxygen saturation level for severely impaired SS cell deformability.

PROPOSED COURSE: As the first step, we used published data, combined with the model we developed for sickle cell deformability, to estimate the shift in the critical oxygen saturation level in SS cell crisis due to the heterogeneity in hemoglobin concentration. We are developing a filtration system to generate data of SS cell deformability under the influence of different intracellular hemoglobin concentrations that will help us in the analysis of our model.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10380-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Pathophysiology of Syringomyelia

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Alec Eidsath, Ph.D.	Staff Fellow	MES, BEIP, NCRR
Robert L. Dedrick, Ph.D.	Chief	CHES, BEIP, NCRR
Thomas Talbot, M.S.	Mechanical Engineer	ACES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
SNB, NINDS (E. Oldfield, J. Heiss)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
  _(a1) Minors
  (a2) Interviews
_(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Syringomyelia is a cavitation of the spinal cord. It most commonly occurs in association with structural abnormalities of the foramen magnum. We plan to assist SNB in the acquisition of pressures in the ventricle, syrinx, and spinal subarachnoid space. The data will be evaluated in connection with anatomic information obtained by cerebrospinal MRI, ultrasonography, and Imatron CT. Correlation of the anatomic and physiologic measurements should help to elucidate the mode of progression of syringomyelia, and may have implications for its optimal treatment.

OBJECTIVES: This study seeks to determine the pathophysiology of the induction and progression of syringomyelia. Establishing the pathophysiology of this disease may have implications for its treatment.

METHODS EMPLOYED: A preoperative series of tests will be conducted in which pressures from the lumbar and syrinx, along with the EKG trace, will be taken while the subject is awake. Pressures will be monitored during modified Queckenstedt tests (a cuff placed around the neck causing a transient increase in the intracerebral pressure), as well as during such common events as coughing and straining. During surgery, measurements will be obtained at three locations along the spine (the lumbar space, syrinx, and lateral ventricle) as well as the main arterial and venous pressures. Data from the pressure transducers will be digitized by means of a general-purpose, analog-to-digital board controlled by a "virtual instrument" written in the LabView programming language. LabView was chosen because it gives an easy-to-use graphical interface to the data collection procedure, which will be essential if it is to be used by technicians in the operating room.

SIGNIFICANCE: The natural history of syringomyelia is one of gradual, progressive deterioration over many years. One objective of surgical treatment is to arrest the disease's progression.

PROPOSED COURSE: One patient has already been seen, and we are in the process of analyzing the data. One problem that must be addressed is how to automate the analysis of the large amount of data taken for each patient. Additional patients will be studied.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10381-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Patient Electronic Monitoring System, Model II

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horace E. Cascio, BSEE	Electronics Engineer	EEES, BEIP, NCRR
George L. Hemphill, BA	Engineering Technician	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
OD, CC (N. Kelly)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
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NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.65	0.25	0.4

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects _(b) Human tissues (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Clinical Center has requested that the BEIP install two additional Patient Electronic Monitoring Systems (PEMS) on the 5E and 3E Nursing Units. A system that alerts the nursing staff when a patient (or patients) is leaving the nursing unit is a welcome aid in the care of patients with Alzheimer's disease or other dementias. The system is based on a wristwatch-size radio transmitter worn by the patient. A detector unit, placed at the two exit doors, senses the presence of any patient wearing a transmitter watch. With the detection of a patient's transmitter, a microprocessor based controller locks the exit door, activates an alarm, and sends the patient's name and location to a personal computer, which displays and records the time and date of each attempt a patient makes to leave the unit.

OBJECTIVES: To redesign the PEMS to include state-of-the-art technologies in order to increase system reliability and reduce maintenance.

METHODS EMPLOYED: An improved, printed-circuit patient identifier will be developed using surface-mount integrated circuits and chip resistors and capacitors. The circuit of the identifier will incorporate a crystal-controlled modulator circuit and will be powered by a rechargeable lithium battery. These features will practically eliminate the weekly maintenance routine. The control unit will use an embedded CPU card that allows software program development on a personal computer, which in turn will eliminate dependency on an obsolete Intel MDS Program Development System. All of the circuitry for the controller will be placed on printed circuit boards in a modular form.

SIGNIFICANCE: The redesign of the PEMS will reduce the need for technical assistance, and permit hardware fabrication by outside contractors at a low cost.

PROPOSED COURSE: The PEMS for the 5E Nursing Unit was built first, since the 5E Nursing Unit was the first unit to be renovated. The renovators have installed all cabling and special devices required for system operation. The evaluation of the new printed-circuit identifiers and the printed-circuit modules are currently under way. The fabrication of the PEMS for 3E will begin in September 1992.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10382-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Walking Speed Indicator

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Horace E. Cascio, BSEE	Electronics Engineer	EEES, BEIP, NCRR
Thang Q. Pham	Engineering Technician Aide	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
EDBP, NIA (J. Guralnik)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.15	0.15

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects _(b) Human tissues (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Impaired mobility is an important consequence of several common chronic diseases of aging, and is a risk factor for falls, hip fractures, and loss of independence. For a newly developed field study on disability in older persons, an accurate portable instrument is required to measure the walking speed of subjects in their homes. The patient's walking speed is measured at one meter and at either three or four meters. Data obtained from this study will aid in the identification of diseases, since different walking patterns can identify different diseases. Those with Parkinson's disease and certain other problems have a distinctive pattern: They begin walking very slowly, but then attain normal walking speed.

OBJECTIVES: To design a lightweight, portable instrument that, once initiated, will operate automatically to measure walking speeds at two distances. The operator will be free to help the subject during the test.

METHODS EMPLOYED: An ultrasonic ranging system, available from Polaroid Corporation, will give instantaneous distance measurements over the four-meter course. Additional circuitry will be developed to detect when the subject reaches the two distances of interest. The times for these distances are stored and displayed on two LCD displays. The instrument will be mounted on a tripod and positioned at the starting line, while aiming at the subject's back.

SIGNIFICANCE: With this portable instrument, one licensed practical nurse could go into many homes and perform the required tests with no help.

PROPOSED COURSE: A prototype has been fabricated and tested. The instrument is now under evaluation by EDBP. Once the evaluation is complete, the instrument will be sent to Johns Hopkins University for further testing. If the instrument passes all evaluations, a contract will be issued for the fabrication of sixteen instruments.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10383-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Diffusion Coefficient Measurement

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
James Mattiello, Ph.D. Staff Fellow BEIP, NCRR

COOPERATING UNITS *(if any)*
Diagnostic Radiology Research Program, Office of Intramural Research, Office of the Director (J. Frank)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Magnetic resonance imaging and spectroscopy are used to obtain, a measurement of the diffusion coefficient of water protons noninvasively. This apparent diffusion coefficient of water samples is proportional to concentrations of gadolinium and dysprosium (MRI contrast enhancement agents). This investigation studies the magnitude and mechanism of this change in the apparent diffusion coefficient, and the ways this change could be used to measure the concentration of the parametric ions.

MAJOR FINDINGS: For two different gadolinium contrast agents and one dysprosium contrast agent, the apparent diffusion coefficient changed as a function of the concentration. The gadolinium agents had different slopes, compared to the dysprosium curve. The magnitude of this slope would allow for the determination of the agent's concentration in the concentration range of 5 to 0.05 mMol.

PROPOSED COURSE: This investigation of the apparent diffusion coefficient change for various concentrations of parametric and nonparametric agents is continuing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10384-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Contribution of Diffusion to Magnetization Transfer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
James Mattiello, Ph.D. Staff Fellow BEIP, NCRR

COOPERATING UNITS *(if any)*
Laboratory of Cardiac Energetics, NHLBI (R. Balaban); Diagnostic Radiology Department, Clinical Center (D. LeBihan)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Magnetization transfer contrast (MTC) is a technique that improves the contrast of magnetic resonance images by applying low-power radio-frequency irradiation, which is 10 kHz away from the main water proton resonance frequency, to excite macromolecular hydrogens. To determine if this rate of magnetization transfer is limited by diffusion of water protons, the temperature dependence of both diffusion and magnetization transfer were determined in the same material. Experiments were conducted on a 4.7-T GE CSI spectrometer; the temperature variation in the material was accomplished by circulating temperature-controlled water through pads surrounding the sample. Spin-lattice relaxation times (T1) were determined with and without the RF saturation pulse. The rate of magnetization transfer for the material was then determined.

MAJOR FINDINGS: T1 without the saturation pulse and diffusion have a linear dependence as a function of temperature over the range observed (10° to 60°C). The magnetization transfer rate was constant over the temperature range of 25° to 45°C, but deviated from this constant value at lower and higher temperatures. The temperature dependence between magnetization transfer and diffusion suggests that diffusion may be a rate-limiting factor in magnetization transfer over the physiological temperature range (25° to 45°C).

PROPOSED COURSE: Further studies on the interaction between diffusion and magnetization transfer are under investigation by Dr. Balaban's group.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10385-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Echo-Planar Diffusion Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

James Mattiello, Ph.D.	Staff Fellow	BEIP, NCRR
Robert F. Bonner, Ph.D.	Physicist	BEIP, NCRR

COOPERATING UNITS *(if any)*
Laboratory of Cardiac Energetics, NHLBI; Diagnostic Radiology Department, Clinical Center (D. LeBihan); Division of Intramural Research, NINDS

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.05	0.05	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Echo-planar imaging (EPI) allows for the acquisition of a magnetic resonance image in less than 0.1 sec. This fast imaging sequence would allow for monitoring in studies in which the time development of a process is important, such as thermal diffusion in a volume. Because it is sensitive to thermal changes, EPI with diffusion gradients will permit rapid imaging of these processes. We are continuing the work of Dr. Robert Turner (formerly with the BEIP, NCRR, and now with the Laboratory of Cardiac Energetics, NHLBI) to develop this EPI diffusion system on the 4.7-T MRI system at the *In Vivo* NMR Research Center.

MAJOR FINDINGS: Due to problems in the 4.7-T hardware that will not allow the gradient to switch on/off at the fast rate necessary for EPI, this project has developed slowly.

PROPOSED COURSE: The problems with the hardware are being worked out; when they have been corrected, work on this project will continue.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10386-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Calculation of Electrical Activity in Cardiac Tissue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Bradley J. Roth, Ph.D.	Staff Fellow	BEIP, NCRR
Joshua M. Saypol	Summer Student	BEIP, NCRR

COOPERATING UNITS *(if any)*
Laboratory of Biophysics, NINDS (J. Clay, V. Kowtha)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.15	0.10

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  _(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A numerical simulation has been performed of the propagation of an action potential through cardiac tissue. The bidomain model was used to account for the effect of both tissue anisotropy and the interstitial space on current flow. A relaxation technique was used to solve the nonlinear partial differential equations.

The model has been modified to predict propagation in a two- or three-dimensional tissue, due to a point source of current. The influence of unequal anisotropy ratios of the intracellular and extracellular conductivities is being investigated. Pairs of stimulus pulses are applied to a two-dimensional sheet of tissue to create rotors and other unusual phenomena, which could prove useful as models of cardiac arrhythmias and fibrillation. The interaction of cardiac tissue with a uniform electric field was also examined.

OBJECTIVES: To increase our understanding of the electrical properties of cardiac tissue, and to provide a mathematical model that can be used to study cardiac arrhythmias.

SIGNIFICANCE: Cardiac arrhythmias and fibrillation are among the most common causes of death among Americans. In order to understand these pathologies, a basic knowledge of the heart and the electrical properties of cardiac tissue is required. In this study we investigate idealized, yet important, models that reproduce many of the experimental data observed in cardiac tissue.

MAJOR FINDINGS: The intracellular and extracellular anisotropies have a major influence on the electrical properties of cardiac tissue. Tissues with "unequal anisotropy ratios" can lead to anodal stimulation and to arrhythmias generated by two pulses passed through a single electrode. Cardiac tissue can be depolarized by a uniform electric field both at the heart surface and deep within the tissue bulk. None of these phenomena could be observed without taking into account the different anisotropies inside and outside of the cells.

PROPOSED COURSE: The properties of the arrhythmia produced by stimulation of the tissue will be characterized in detail, and ways to terminate the arrhythmia will be studied. The interaction of a spherical heart with a uniform electric field will be analyzed both analytically and numerically.

PUBLICATIONS: Roth B. How the anisotropy of the intracellular and extracellular conductivities influences stimulation of cardiac muscle. J Math Biol (in press).

Roth B. A comparison of two boundary conditions used with the bidomain model of cardiac tissue. Ann Biomed Eng 1991;19:669-78.

Roth B, Saypol J. The formation of a re-entrant action potential wave front in tissue with unequal anisotropy ratios. Int J Bifurcation and Chaos 1991;1:927-8.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10387-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A Three-Dimensional Motion Measurement System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Horace E. Cascio, BSEE Electronics Engineer EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
BL, RM, CC (S. Stanhope); Adtech (A. Dainis)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.35	0.25	0.1

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The Biomechanics Laboratory makes quantitative information concerning human motion available to clinicians and researchers at the NIH. The BEIP has upgraded Dr. Stanhope's Vicon Motion and Image Analysis System by designing and fabricating six video-camera units, each with a variable intensity dual-wavelength infrared strobe. The upgrade solved many of the original Vicon system's problems, but fast motion analysis is still limited by the system's slow frame rate of 25 frames per second. The Biomechanics Laboratory requires a system to take data at 2000 frames per second. To satisfy this requirement, Dr. Stanhope requested that the BEIP develop a three-dimensional motion measurement system, using a novel idea patented by Dr. Andrew Dainis. The terms of the CRADA state that the NIH will develop a clinical operating 3-D motion analysis system; in return, the NIH will keep the system. Dr. Dainis will have all rights to the BEIP's part of the design. In this venture with the Biomechanics Laboratory and Dr. Dainis, the BEIP will develop, fabricate, and test the system electronics. Dr. Dainis will develop the optics for the CCD linear arrays and the system software. The idea of the patent is that each camera will have four linear CCD arrays, with each array having a specially designed lens. The arrays are placed on the face of the camera in a special pattern to translate a light source from a target into its spatial coordinates.

OBJECTIVES: To design and fabricate a three-dimensional motion and image analysis system under a CRADA. The system will consist of several freestanding cameras, data acquisition and interfacing circuitry, and a controlling computer. A major goal of this project is to obtain a system with frame rates to 2000 Hz.

METHODS EMPLOYED: The system consists of one to sixteen cameras, a system controller, and a personal computer. The camera will contain signal processing, on-board memory, and interfacing circuitry to the system controller. The sensors are four 2048-element CCD linear arrays with a maximum sweep speed of 10 MHz. Frame rates near 5 KHz can be obtained, but a frame rate of 2 KHz would be acceptable. The cameras view targets located on a patient. The illumination of these targets can be active (LEDs or incandescent lamps) or passive (reflective spheres). A system controller will receive instructions from the host computer, and will then control the data acquisition session between all cameras. The cameras will be under computer control except when collecting data, when they will operate independently. The system controller releases the host computer for performing other real-time functions. A personal computer (PC) sets up the data acquisition procedures, then records real-time analog information from different sensors. At the completion of the testing session, the PC reads, analyzes, and displays the data from each camera in an appropriate form. For a multicamera system with requirements to collect position coordinates of many targets at a fast frame rate and to collect real-time analog information, the use of parallel CPUs adds to system efficiency and reliability. The 486 processor in the PC and a 186 processor on the embedded CPU card in the system controller give this system dual processing.

SIGNIFICANCE: A three-dimensional motion analysis system that can perform studies at high frame rates would be a welcome addition to the Biomechanics Laboratory, especially in studies of lip and tongue motions.

PROPOSED COURSE: To design and fabricate one camera unit and the system controller, and to test and evaluate the operation of the hardware.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10388-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
3-D NMR Imaging

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ching-Nien Chen, Ph.D. Physical Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A new 3-D NMR imaging technique is under investigation. The technique needs an extra set of magnetic field gradients to encode 3-D information. A homebuilt RF probe and gradients for a small test sample were constructed. Preliminary phantom studies have been done.

PUBLICATION: Delannoy J, Chen C-N, Turner R, Levin RL, LeBihan D. Noninvasive temperature imaging using diffusion MRI. Magn Reson Med 1991;19:333-9.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10389-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Data Processing of Fluorescence Microscope 3-D Images

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Ching-Nien Chen, Ph.D. Physical Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
Johns Hopkins University (S. Lesko)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
In Vivo NMR Research Center

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

X _(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Fluorescence probes are designed and used to mark specific chromosomes in cells cultured from human fibroblasts. 3-D microscopic images obtained therefrom are used to study the spatial distribution of the chromosomes. They are generally regarded as randomly distributed in the cell. Images taken at certain stages of cell division are reoriented according to some preset rules, then analyzed to see if there is any degree of order existent. Preliminary studies showed that there is indeed some order for the three kinds of chromosomes (No. 11, No. 17, and No. 21) that were probed. This finding may be significant to the understanding of cell division mechanisms.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10390-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Simultaneous Voltametry, Raman and Absorption Spectroscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
LCP, NIDDK (I. Levin, P. Harmon); LC, NHLBI (R. Hendler)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Raman and multiwavelength optical absorption spectroscopies and potentiometric measurements are standard techniques, used individually to study proteins and other biological molecules. This instrumentation permits the redox state of the protein solution to be titrated using the potentiometric technique with simultaneous recording of the Raman and absorption spectra associated with each state.

OBJECTIVES: To design and construct an optical flow cell providing control of solution flow, control of the redox potential of the solution, and access for both Raman and optical spectroscopy. To develop instrumentation for controlling the potentiometric titration and for collecting the multiwavelength optical absorption spectroscopy.

METHODS EMPLOYED: An optical flow cell was designed and fabricated to permit protein (or other biological) solutions to be circulated within the cell. The cell had the following features: Potentiometric electrodes were provided to enable the redox.potential of the solution to be established; optical windows were provided to permit the solution's Raman and optical absorption spectra to be obtained.

An optical system was designed to provide a uniform spatial light field from a stable incandescent light source (Newport Corporation). The transmitted light was focussed on the entrance slit of a monochromator attached to an optical multichannel analyzer, OMA (Oriel Corporation). The data gathering is controlled by a PC-based computer, which has several data processing alogorithms to analyze the data.

The potentiometric measurements and control were implemented digitally with the aid of a commercial analog-to-digital/digital-to-analog card added to the computer that is part of the Oriel OMA, plus appropriate software.

SIGNIFICANCE: The simultaneous gathering of potentiometric, Raman, and absorption data on the same solution is a powerful tool in the study of proteins. Potentiometric control enables these studies to be performed at known redox states of the protein.

PROPOSED COURSE: The instrumentation has been constructed and is performing as designed. A series of biological studies is underway to correlate the spectra obtained by Raman and absorption spectroscopies.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10391-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Optical Fiber Coupler for Microscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR
James V. Sullivan	Acting Section Chief	MIFS, BEIP, NCRR

COOPERATING UNITS *(if any)*
LKEM, NHLBI (K. Spring)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.75	0.50	0.25

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A uniform illumination field is necessary to enable sophisticated image processing techniques to be applied to quantitative microscopy. Achieving this uniformity is possible with a single illumination source, but it becomes tedious if more than one source is used to excite different chromophores within the sample of interest. A 3-to-1 optical coupler was designed and constructed to permit selection of three different sources for introduction into the microscope without disturbing the critically aligned input optical fiber to the microscope illumination port. Sources may be employed in the ultraviolet down to 250nm.

OBJECTIVES: To develop an optical system to allow more than a single illumination source to be used for a microscope. The system must permit selection to be made with minimal disturbance to the uniformity of the microscope's observation field.

METHODS EMPLOYED: The differences in construction of illumination sources (e.g., the size and intensity profiles between a thermal incandescent and a xenon arc) impose constraints on an optical system designed to provide uniform illumination at the observation field of a microscope. This is especially true when rapid switching is required between illumination sources.

An optical fiber approach was selected to overcome these limitations. A single fiber had previously been successfully employed to provide uniform illumination from a laser source. (Initial attempts to bundle three 200-micron-diameter fibers into a single source failed, because the spatial geometry of each fiber source creates three optical axes into the microscope.) The optical design requires that the final source fiber be situated permanently on the microscope's input optical axis, and that the three input fibers from each of the individual light sources be coupled into the final fiber. The overall length of the fibers must be sufficient to allow mode-mixing to occur along the fiber length. Typically, 3-m lengths of 600-u fused silica, solid-core optical fibers have been used.

Mechanically, the three input fibers are tapered at one end and cleaved perpendicular to their axis in such a way that the final diameter is 283u. All other fiber ends are cleaved perpendicular to the axis. The three input fibers are brought together and abutted against the input end of the microscope fiber, and permanently held in place using an axial sleeve made of stainless steel. Index matching fluid is interposed between the ends of the fibers forming the coupler to increase transmission.

Efficiencies in excess of 75% for each arm of the optical fiber coupler have been achieved.

SIGNIFICANCE: Preservation of the illumination field uniformity between differing sources is an important parameter when digital image processing is performed on microscope images captured with each illumination source. Ultimately, it limits the resolution of small details contained within the images.

The optical coupler can also be used in reverse to split a beam into three components. The tapering technique is not limited to a 3-to-1 geometry; other configurations (for example, 4-to-1 and 2-to-1) are possible. Further, other materials and diameters of optical fibers should also be appropriate.

PROPOSED COURSE: A redesigning of the mechanical support for the device is being pursued to improve its reliability and robustness. A patent application has been filed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10392-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Remote Laser Control for Photodynamic Therapy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR
Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR
John Cole, MSEE	Electronic Engineer	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
ROB, DCT, NCI (A. Russo, T. Delaney); SURG, DCT, NCI (H. Pass)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
X _(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Photodynamic therapy (PDT), which is the interaction of light with hematoporphyrin derivative (HPD or Photofrin II) in tissue, is in clinical use in several areas of application. Currently, PDT is being used clinically for bronchial obstructions, carcinoma *in situ* of the bladder, and intraperitoneal and pleural tumors. The treatment light is provided by two high-powered argon ion/dye lasers, each capable of operating at 514nm or 630nm, and at two delivery levels. Since the lasers have RS-232 interfaces for control and monitoring, a computer makes an ideal device for remote control from the operating room. The computer can also provide timing for each segment, a permanent log of level versus time, and integrated energy delivered. It also allows the individual operating the light monitoring system to control the illumination.

OBJECTIVE: To develop a computer-based remote control system for the two lasers used for photodynamic therapy on human patients.

METHODS EMPLOYED: A small laptop computer is connected to each laser via a two-channel RS-232 interface box. A program has been written that allows this computer to control the functions of both lasers, and to read the current status of various parameters from each. The program stores the laser powers at regular intervals, the time of each change in laser state, significant times and energy levels, and user-entered comments.

MAJOR FINDINGS: This system provides a very convenient and flexible method for controlling the laser energy levels from the operating room at the site of the procedure.

SIGNIFICANCE: Fewer personnel are now needed during a PDT procedure; the surgeon-requested changes in laser condition are made much more quickly; the system operator in the operating room has a much clearer indication of what the lasers are doing at any time during a procedure; and there is a permanent record of both the status of each laser and the changes in operating conditions throughout the procedure.

PROPOSED COURSE: Further software developments will be made as new requirements arise during use for both current and new types of treatment.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10393-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Thermal Cycling of Incubator Baths for Polymerase Chain Reaction

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
ROB, DCT, NCI (A. Russo)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.0	0.5

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The polymerase chain reaction has rapidly become an indispensable tool of molecular biology. For new agents, searching for the most effective thermal cycle for lysis, quenching, and annealing of the nucleotide is a time-consuming process. The instrumentation being developed will streamline the search process by automatically cycling the reactants through predetermined temperature profiles. The technology employs PC-based computer control of five independent thermoelectric modules, and is readily expandable to greater numbers of reactant chambers.

OBJECTIVES: To develop thermal cycler instrumentation for searching and establishing the optimal conditions for new reagents used in the polymerase chain reaction. The instrumentation must perform independent ramp and soak temperature cycles for each of five reaction chambers.

METHODS EMPLOYED: Thermoelectric (Peltier) modules are capable of heating or cooling an element, depending on the direction of current flow through the module. The PCR reaction chamber, holding 200 ul of reactant, is mounted in a copper housing to which two 19-watt thermoelectric modules are attached on opposite faces. The outer face of each module is attached to a water-cooled heat exchanger.

A typical thermal cycle calls for a temperature sequence of 50C to 90C to 70C and back to 50C. Ramping the temperature between these set points must be achieved in 30 seconds or less. The reaction chamber is held at each set point for times between 30 seconds and 2 minutes. Each set point must be reproducible in subsequent cycles and must be accurate to 1C. A prototype single well set-up showed the thermoelectric modules easily achieved both the ramping and the set-point requirements, using an auxiliary water circulator at 50C for the heat exchanger.

Control of the thermal cycles is achieved by a program running on a desktop PC. The program will use PID techniques to drive the thermal elements in a manner that will provide the required rapid temperature jump. The thermal elements will be driven by power-operational amplifiers, and the temperature will be measured by platinum RTD devices. The control program will allow selection of parameters for each well, and will display the current set-points for each active well, as well as a plot of the progress of each.

SIGNIFICANCE: The polymerase chain reaction is an indispensable technique of molecular biology. This development will significantly reduce the time spent on searching for the optimum temperature cycle associated with new reagents as they become available. The concepts employed in this designed are not limited to the prototype five-well system being developed, but are readily expandable to larger systems.

PROPOSED COURSE: To complete design and construct the interface hardware; to finish writing and to debug the control and data acquisition programs; to determine optimal PID constants; and to test and deliver the final operating system.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10394-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Biological Pulsed Electronic Spin Resonance System

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR
Thomas J. Pohida, BSEE	Electronic Engineer	EEES, BEIP, NCRR
Paul D. Smith, Ph.D.	Physicist	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
ROB, NCI (J. Mitchell, A. Russo, J. Bourg, M. Krishna, S. Subrumanian); LCP, NIDDK (R. Tschudin); CSL, DCRT (H. Fredrickson)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.4	1.2	0.2

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

For several years, nitroxides have been a major focus of research in ROB, NCI because of their importance to radiation biology in general and their potential utility for new photodynamic therapy techniques. Electron spin resonance (ESR) is a powerful tool for free radical studies, but in the past has been of limited value for biological work (especially *in vivo*) because of the excessive attenuation by water of the radio frequencies ordinarily used, about 9GHZ. The system now being developed will operate at about one-thirtieth of that frequency to alleviate the attenuation problem, and will incorporate pulse techniques developed in NMR, as well as other new techniques, to compensate for the inevitable thirtyfold loss of sensitivity.

OBJECTIVES: To develop a pulsed ESR apparatus optimized for the study of nitroxides and other compounds of interest under *in vivo* conditions. Initial measurements will be spectroscopic, but imaging will be subsequently undertaken.

METHODS EMPLOYED: The initial configuration will comprise a set of Helmholtz coils, a probe, a pulse programmer, a radio frequency system, data acquisition and processing circuitry, and a PC (or perhaps two PCs) for overall system control and data handling. All timing increments will bear exactly the same ralationship to the radio frequency carrier on successive excitations. New excitation techniques are under study to provide the broad bandwidth needed for nitroxides (approximately 150MHZ) without requiring excessive peak power. EEES personnel are concentrating particularly on the design and construction of a custom high-speed averager. Commercially available averaging units are not fast enough for these measurements--a limit imposed by the very short relaxation time of electrons compared to nuclei. This unit will average 1000 free-induction decays, and will transfer the data to the computer in about 5ms. The computer can further average 1000 such transfers, resulting in the averaging of 1,000,000 decays in about five seconds, with a thousandfold (or 60-decibel) improvement in signal-to-noise ratio, which will help compensate for the low sensitivity resulting from operation at a low frequency and other factors. Helmholtz coils have been designed and constructed to provide a uniform magnetic field throughout a volume (5 x 5 x 10cm) in their center.

SIGNIFICANCE: Achievement of the proposed specifications will greatly facilitate research on nitroxides and other compounds of interest in biological systems.

PROPOSED COURSE: Design of the high-speed averager is nearly complete, and fabrication is under way. Most other system components have been procured; detailed design is in progress.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10395-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Digital Differential Thermistor Thermometer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Walter S. Friauf, MEE	Section Chief	EEES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electronic Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(If any)*
LBC, NHLBI (R. Berger)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electrical and Electronic Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.7	0.5	0.2

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Full exploitation of the potential of differential calorimetry requires small sensors with rapid response and high sensitivity, virtually limiting the choice to thermistors. The small size is necessary so that the sensors can be very close together to avoid masking a minute difference in heat of reaction by extraneous temperature gradients. For convenient use by laboratory and health-care personnel, the apparatus must be fully automated.

OBJECTIVES: To develop a fully automated differential thermistor bridge with high speed, sensitivity, and common mode rejection.

METHODS EMPLOYED: A personal computer (PC) with a standard plug-in interface board was used to provide the automation features, as well as data storage and display. A custom bridge configuration was designed to allow the computer to control balancing without the usual adjustment of the resistance of one arm, which is impossible with any standard interface board. The computer also controls bridge excitation to set the power level and allow sensing and correction of all thermoelectric potentials and amplifier offsets, as well as linear time variance of both.

SIGNIFICANCE: Achievement of the desired objectives will allow the construction and use of small probes with a suitable coating sensitive to the concentration of a specific compound on one thermistor. The instrument is also useful for general differential calorimetry, particularly when very fast response is needed.

PROPOSED COURSE: The instrument has been designed and built, and performs very near the theoretical limits of speed and sensitivity imposed by the Johnson noise of the thermistors. It is now undergoing evaluation in several experimental applications. The development of specific compound probes will be undertaken soon. Circuit analysis has disclosed that departure from perfect balance affects thermal common mode rejection, so that a slight imbalance can be used to compensate for imperfect matching of the temperature coefficients of the two thermistors. If necessary, the software needed to effect this imbalance will be developed.

PUBLICATION: Friauf WS, Clem Sr TR, Sr., Berger RL. A personal computer-controlled differential thermistor bridge. In: Proceedings of the seventh international symposium on temperature. Amer Inst of Physics, 1992 (in press).

PATENT: A patent application has been filed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10396-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Characterization of Frozen-Hydrated Specimens by EELS

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	BEIP, NCRR
Shanling Shi, M.S.	Visiting Associate	BEIP, NCRR

COOPERATING UNITS *(if any)*
LN, NINDS (S. B. Andrews)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A method has been developed for measuring water content in subcellular compartments of frozen hydrated tissue that depends on variations in the valence excitation energy loss spectrum between water and the organic constituents of a cell. Single-scattering distributions are first obtained from ice and protein by applying the Fourier-logarithmic deconvolution technique. The resulting reference spectra are then normalized by applying the Bethe sum rule to the differential oscillator strengths in order to derive the differential cross sections. A direct estimate of the water content can be obtained by applying a least squares fitting algorithm to spectra from hydrated specimens. Tests have been performed on thin cryosections of rapidly frozen bovine serum albumin standards, containing 70%, 80%, and 90% protein, by recording parallel-EELS spectra in the field-emission scanning transmission electron microscope (STEM). It was found that an accuracy of ~1% could be achieved in the determination of water content. The method has been applied to cryosectioned erythrocytes and cerebellar cortex.

OBJECTIVES: To develop a direct method for measuring water content of subcellular organelles by analyzing the valence electron spectrum; to establish how the accuracy, precision, and spatial resolution are limited by radiation damage; and to apply this approach to measure water distributions in brain and other tissues.

METHODS EMPLOYED: EELS data were recorded with a VG Microscopes field-emission STEM, equipped with a cryotransfer stage and a Gatan parallel-detection electron energy-loss spectrometer. Specimens were prepared by quick-freezing against a liquid nitrogen-cooled copper block, and then cryosectioning, depositing sections onto grids, and cryotransferring into the STEM. Spectra were acquired with the Gatan parallel-detection electron energy-loss spectrometer, which was de-scanned to provide optimal energy resolution (0.4 eV) from extended areas of the specimen. The spectra were processed by means of special programs developed with MatLab software on the Macintosh II.

MAJOR FINDINGS: Inelastic scattering cross sections for water and protein have been obtained by using the Fourier-logarithmic deconvolution to derive the single-scattering distribution, and the Bethe sum rule to calculate the differential oscillator strength. The water spectrum is characterized by a band-edge at 9.1 eV and a plasmon peak at 20.4 eV, whereas the protein exhibits a peak at 6.7 eV (attributable to an excitation of the amide bond) and a plasmon peak at 23.5 eV. Results provide the first reliable estimate for the inelastic mean free path of water as 195±15 nm (at 100 keV beam energy). Measurements on rapidly frozen solutions of bovine serum albumin containing 70%, 80%, and 90% protein demonstrate that an accuracy of ±1% in the water content can be achieved. The estimated concentrations are in good agreement with the nominal values. The precision depends on the spatial resolution due to radiation damage effects, but it is feasible to obtain useful data on the scale of 100 nm. Preliminary measurements made on rapidly frozen and cryosectioned erythrocytes, as well as on cerebellar cortex, give results that are in the expected physiological range.

SIGNIFICANCE: Water measurements on ultrathin cryosections using x-ray continuum measurements are generally performed indirectly after freeze-drying, because the presence of water renders the specimens highly susceptible to radiation damage at high dose. The availability of a direct approach that could be applied at low dose to frozen-hydrated specimens would therefore provide more reliable (as well as new) information. The subcellular water distribution is an important quantity that reflects the physiological processes in the cell (e.g., passive water regulation by ion channels or active regulation by water-specific channels). The water content must also be known in order to convert the dry weight concentrations of ions, obtained from x-ray microanalysis, to the physiologically more relevant molar concentrations. There are also a number of potentially important

applications of water mapping to pathology, including measurement of changes that occur in brain injury.

PROPOSED COURSE: To extend the approach by obtaining two-dimensional water distributions with the EELS spectrum-imaging technique. Low-loss spectra will be acquired at each pixel, and the data will be processed *post facto* in order to correct for spectrum drift, deconvolute plural inelastic scattering, correct for the support film, and fit reference spectra to determine the water content.

PUBLICATIONS: Sun S, Leapman RD. Characterization of frozen hydrated biological specimens by low-loss EELS. In: Bailey GW, ed. Proceedings of the 50th annual meeting of the Electron Microscopy Society of America. San Francisco: San Francisco Press, 1992;1572-3.

Sun S, Shi S, Leapman RD. Measurement of water content in frozen-hydrated biological specimens by low-loss EELS. Ultramicroscopy (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10397-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Subcellular Composition of the Pancreatic Islets of Langerhans

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Richard Leapman, Ph.D.	Physical Scientist	BEIP, NCRR
Shanling Shi, M.S.	Visiting Associate	BEIP, NCRR
Stanley Sun, Ph.D.	Visiting Fellow	BEIP, NCRR

COOPERATING UNITS *(if any)*
LCBG, NIDDK (I. Atwater)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Electron Beam Imaging and Microspectroscopy Group

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Rapid-freezing and cryosectioning techniques have been established for obtaining specimens from isolated, incubated rat pancreatic islets of Langerhans. The existing freezing machine was modified to function at liquid nitrogen temperatures, so that it was possible to freeze individual islets mounted with physiological buffer on a gold freezing 'hat.' The cryoultramicrotome was set up with a diamond trimming tool, a 35° diamond knife, and a 12-kV antistatic gun to prevent the tissue from charging during cryosectioning. With these modifications, it is now possible to obtain high-quality cryosections on which useful experiments can be performed, both in the Hitachi TEM-STEM and in the VG Microscopes field-emission STEM.

OBJECTIVES: To investigate ionic changes that occur in the secretion of insulin and glucagon by the pancreatic islet beta and alpha cells, respectively; and to determine what role these organelles play in regulating calcium. Of particular interest is the effect of glucose stimulation on the calcium concentrations.

METHODS EMPLOYED: Isolated islets were rapidly frozen against a liquid nitrogen-cooled copper block, mounted in a cryoultra-microtome, and sectioned to a thickness of ~100 nm. Sections were deposited on carbon-coated grids, and were cryotransferred into either a TEM-STEM, equipped with a conventional thermionic source, or into the VG Microscopes STEM, equipped with a field-emission source.

MAJOR FINDINGS: Cryosections have been cut successfully from rapidly frozen islets. By modifying the microtome, it has been possible to obtain uniform ribbons of cryosections similar in appearance to conventionally embedded material. Although this cryosectioning technique is a difficult one, it is a prerequisite for obtaining useful analytical data. Moreover, we have shown that it is essential to use a diamond (rather than a glass) knife to section pancreatic islets. Having achieved this important first step, we are now in a position to obtain analytical data.

SIGNIFICANCE: Electron probe x-ray microanalysis of pancreatic islets of Langerhans allows variability to be assessed, as well as averages of granule elemental composition. For the first time, we have been able to obtain specimens with good structure, which should allow unambiguous identification of organelles. The high sensitivity of the field-emission STEM should provide more precise estimates of the secretory granule composition in cells in different physiological states. Such measurements can be performed *in situ*, rather than on extracted organelles.

PROPOSED COURSE: It is planned to obtain x-ray analytical data on islets that have been stimulated to secrete insulin by incubation of the islets with high glucose.

PUBLICATIONS: Foster MC, Leapman RD, Li MX, Atwater I. Elemental composition of secretory granules in pancreatic islets of Langerhans. Biophys J (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10398-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
NMR Diffusion Imaging and Spectroscopy

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.)* *(Name, title, laboratory, and institute affiliation)*

Peter Basser, Ph.D.	Senior Staff Fellow	BEIP, NCRR
James Mattiello, Ph.D.	Staff Fellow	BEIP, NCRR

COOPERATING UNITS *(if any)*
Diagnostic Radiology Department, Clinical Center (D. LeBihan)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.8	0.8	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

We relate the diagonal and off-diagonal elements of the effective self-diffusion tensor, D, to the echo intensity in NMR spin-echo experiments. This relationship is used to design pulse sequences from which D can be estimated. This estimate is validated using both isotropic and anisotropic media. One potential application of this work is to use D to determine fiber orientation *in vivo*. Specifically, the components of D are used to infer fiber orientation, mean diffusion distances, and tissue microstructure within a voxel. D is used to construct a diffusion ellipsoid in a voxel that depicts the local fiber orientation and mean diffusion distances. The eigenvectors and eigenvalues of D are its principal axes and principal diffusion coefficients. Three scalar invariants of D that are independent of the reference frame in which both magnetic field gradients and D are measured, provide information about tissue microstructure that can be imaged in biological tissue and other anisotropic media.

OBJECTIVES: To increase our understanding of the microscopic milieu in various biological tissues by tracking the diffusion of protons. To develop clinically applicable methods of measuring molecular displacements of protons and other ions on a micron-length scale in biological media.

SIGNIFICANCE: No known noninvasive method exists to measure proton diffusion *in vivo*, other than NMR. The potential value of measuring the diffusion tensor lies in determining local changes in membrane permeability, monitoring edema, and determining local microstructure of tissue (e.g., its fiber orientation). In anisotropic tissue like brain white matter and muscle, new quantities are proposed to replace the diffusion coefficient that might be measured and monitored in disease. This method may have applications in nondestructive testing, as well.

MAJOR FINDINGS: Preliminary results suggest that the effective diffusion tensor can be estimated in order to infer fiber direction or orientation in skeletal muscle noninvasively within a few percent. Significant errors are made in diffusion NMR spectroscopy and imaging of anisotropic skeletal muscle when off-diagonal elements of $\underline{D}$ are not considered.

PROPOSED COURSE: To extend diffusion tensor estimation from a single voxel to diffusion NMR imaging.

PUBLICATIONS: Basser PJ, Mattiello J, LeBihan D. Self-diffusion in anisotropic media and the NMR signal. SMRM, 1992 (in press).

Basser PJ, LeBihan D. Fiber orientation mapping in anisotropic media using NMR. SMRM, 1992 (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10399-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of a Cell Sorter

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John I. Peterson, Ph.D.	Chemist	BEIP, NCRR
Stephen B. Leighton, Ph.D.	Mechanical Engineer	BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electronics Engineer	BEIP, NCRR

COOPERATING UNITS *(if any)*
MET, DCBDC, NCI (J. Berzofsky)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Chemical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
(a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An instrument is being developed for selecting individual cells from growth medium and placing them in wells in a tray (or some other container) for testing or monoclonal growth. Traditional pipetting of a diluted cell suspension is slow and labor-intensive, and requires further processing to select the samples containing a single cell. Recently, a commercial instrument has become available (Quixell) that attaches to a microscope, and consists essentially of a robotic pipetting system. This instrument has certain manipulative advantages, but requires visual cell selection with manual control. The instrument under development would combine automatic cell selection with computer-controlled cell placement.

OBJECTIVES: The objective of this project is to develop an instrument that can select single cells from a growth medium and place them into wells on a tray.

METHODS EMPLOYED: The first approach is to test the feasibility of electrochemical detection of cells.

SIGNIFICANCE: The selection of single cells by pipetting from a very dilute medium is slow and tedious (although automatable), and is based on a statistical expectation of getting mostly single cells placed into wells. Further testing and evaluation must be done to discard wells with either no cells or more than one cell. The instrument envisioned would be completely automatic in cell selection and placement, and would be of considerably greater value than a commercially available instrument that places cells robotically but requires visual-manual selection.

MAJOR FINDINGS: The project has just begun, and the electrochemical approach being tested for cell selection has experienced difficulty with excessive signal noise. An optical selection system is being considered.

PROPOSED COURSE: Evaluation of the electrochemical approach will continue; if it does not appear to be feasible, an optical cell selection system will be evaluated. If the cell selection system is satisfactory, then work will progress to develop a computer-controlled flow system for diverting individual cells to the desired location.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10401-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Multiresolution Techniques for the Compression of X-Rays

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Michael Unser, Ph.D.	Visiting Scientist	BEIP, NCRR
Akram Aldroubi, Ph.D.	Senior Staff Fellow	BEIP, NCRR

COOPERATING UNITS *(if any)*
CEB, NLM (L. Berman, R. Long)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.25	0.25	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

The purpose of this project is to develop new compression techniques and to study their efficiency for the coding of cervical and lumbar spine x-ray data. We have chosen to concentrate our efforts on multiresolution schemes, which are especially suited for progressive transmission. Our present approach uses a pyramidal image representation that consists of a series of increasingly coarser copies of the original, with a scale reduction by a factor of two at each step. The quantized differences between successive levels in this pyramid are then coded for transmission. The image is reconstructed in a progressive fashion, starting from its coarsest representation. Our contribution has been in the design of an improved reduction algorithm based on the principle of least squares approximation. This approach is, in fact, closely related to the wavelet transform.

OBJECTIVE: There are currently four main objectives to this research: (1) the design of good filter banks for pyramid and wavelet decomposition; (2) the investigation of optimal methods for bit allocation and quantization; (3) the implementation of these algorithms on a UNIX workstation; and (4) comparison with other existing methods (e.g., JPEG cosine transform coding, vector quantization) using x-ray data. The final results will be evaluated by radiologists.

METHODS EMPLOYED: An image is represented by a pyramid in which each level is a coarser (and more compact) copy of the original. The pyramid and wavelet filters are designed to minimize the energy of the difference between two successive levels. These difference images are then quantized and represented by a reduced number of bits. This information is then transmitted to the decoder, and the image is reconstructed progressively, starting from the coarsest representation. The least squares pyramid decomposition algorithm has been coded in C, and soon will be ported on a UNIX image processing workstation. These algorithms will be tested using the DXPnet database, which consists of a large collection of digitized x-rays of the cervical and lumbar spine.

MAJOR FINDINGS: Our preliminary results indicate that our least squares design technique provides a substantial performance improvement over the Laplacian/Gaussian pyramid commonly used in this context.

SIGNIFICANCE: These data compression techniques may turn out to be an essential component for the DXPnet project (digital x-ray prototype network). This project is sponsored by NLM, in collaboration with both the National Center for Health Statistics and the NIAMS. This study requires the transmission of a large number of x-rays over *internet* to radiologists for evaluation. Currently, the transmission of a single noncoded image takes about 20 minutes; it is essential to reduce this time lag to a more acceptable level.

PROPOSED COURSE: The next step is to address the important issues of bit allocation and quantization.

PUBLICATIONS:
Unser M. An improved least squares Laplacian pyramid for image compression. Signal Processing 1992;27(2):187-203.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10402-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Processing of Ultrasound Images of the Tongue

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Michael Unser, Ph.D. Visiting Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
CNB, NINDS (M. Hallett); Johns Hopkins Univ. (M. Stone)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.4	0.4	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
_(a1) Minors
_(a2) Interviews
_(b) Human tissues
X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

In this project, we seek to characterize the differences between the tongue movements of normal speakers and those of speakers with cerebellar ataxia, based on the analysis of sequences of ultrasound images. Specifically, the aim is to extract tongue surface features that are both biologically meaningful and statistically useful.

To facilitate and automate this process, we developed a special-purpose image processing system for a Macintosh II personal computer. The conception of this system is modular. The following are the successive processing steps: (1) a prefilter for noise reduction; (2) a geometrical transformation (polar coordinates); (3) an enhancement of the tissue-air interface in the surface region of the tongue by matched filtering; and (4) an extraction of border points by searching for an optimal radial path along the angular dimension. The last-mentioned task is performed very efficiently using dynamic programming. This system can handle image sequences in a fully automatic mode. The procedure was tested using a series of representative tongue profiles; the computer-extracted contours were found to be in very good agreement with the manual tracings of an experienced experimenter.

OBJECTIVES: The goal of this project is to automate the extraction of the tongue surface by developing an image processing system for a Macintosh II personal computer. This task was previously carried out manually, which is quite time-consuming.

METHODS EMPLOYED: The image processing system that has been designed can process sequences of sagittal tongue sections stored in standard TIFF format, and can be digitized using a commercially available framegrabber (Data Translation board). The system is composed of four independent processing modules: (1) a prefilter (smoothing) for noise reduction; (2) a resampling of the sector of interest in polar coordinates; (3) an enhancement of the tissue-air interface in the surface region of the tongue (vertical differentiation); and (4) an extraction of border points by searching for a radial path along the angular dimension. The program has a macro mode in which a predefined sequence of ultrasound images can be processed automatically without any further user interaction.

MAJOR FINDINGS: The tongue surface extraction algorithm has been tested on a set of representative tongue sections. The extracted contours were compared with manual tracings obtained by an experienced experimenter. The observed discrepancy was within the range of the manual intermeasurement variation.

SIGNIFICANCE: The use of ultrasound imaging provides a novel approach to analyzing tongue configurations that may lead to a better understanding of some of the mechanisms underlying speech production. The use of computer techniques can automate and standardize the data extraction part of this process, and can handle long sequences of images.

PROPOSED COURSE: We will apply our system to the analysis of larger data sets, and will study the evolution of tongue configurations over time. We will also improve the program's functionality by adding certain new features (contour editing, tracking of a selected point, feature extraction, etc.).

PUBLICATIONS:

Unser M, Stone M. Automated detection of the tongue surface in sequences of ultrasound images. J Acoust Soc Am 1992;91(5):3001-7.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10403-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Functional Analysis and Applications to Biomedical Image Processing

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Akram Aldroubi, Ph.D.	Senior Staff Fellow	BEIP, NCRR
Michael Unser, Ph.D.	Visiting Fellow	BEIP, NCRR
Murray Eden, Ph.D	Director	BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An important aspect of biomedical data and image processing is to find signal representations that are adapted to the application. The Fourier transform, for example, is well adapted to analyzing the frequency components of signals. Shannon's sampling theory is well adapted to analogue digital conversions. Thus, it is well suited to the processing and storage of signals and images by digital computers. However, Shannon's sampling theory is restricted to band limited functions. We have developed a general sampling procedure for non-band limited signals. In particular, by considering sampling as a problem of approximation in translation-invariant function spaces, we have shown that the least squares approximation of a signal consists of a sampling procedure generalizing the classical one. It consists of an optimal prefiltering, a pure jitter-stable sampling, and a postfiltering for the reconstruction. We have also developed a general theory of representation that uses the new concepts of multiresolutions and wavelets, which are well adapted to multiscale signal processing, edge detection tasks, signal analyses, coding, and compression.

OBJECTIVES: Our goal is to develop transforms that are well suited to different tasks of data processing and biomedical image processing. These transforms should be adapted to problems in signal analysis (such as EEG or ECG signals). They should also be useful for detection tasks and for storage and retrieval of large volumes of data such as MRI images, image series, and 3-D images.

METHODS EMPLOYED: The sampling theory is based on the study of function spaces obtained by weighted sums of a generating function and its translations on the integers. The orthogonal projection of a signal on these spaces is equivalent to a sampling procedure, which consists of a prefiltering followed by a sampling and a postfiltering for the reconstruction. The multiresolution and wavelet theories of representation are similar to the sampling theory in that they are defined using a single function. The linear combination of the translates and dilates of the scaling function generates the multiresolution signal spaces. A similar construction with a wavelet function generates the wavelet spaces. By using convolution of scaling functions, we obtain other scaling functions with more smoothness. Using linear combination of the translates of a scaling function (or wavelet), we construct other scaling functions (or wavelets) with certain desired properties.

MAJOR FINDINGS: The general sampling theory we have derived generalizes Shannon's sampling procedure to non-bandlimited signal spaces. Moreover, we have established the link with Shannon's sampling theory. The convolution sequences of generating functions indexed with n allow us to construct sequences of increasingly smooth signal spaces. We have proven that the corresponding sampling schemes converge to the classical sampling procedure of Shannon as the index n tends to infinity. We have found a simple constructive method for constructing scaling and wavelet functions with some desired properties. For example, we can construct symmetrical interpolating wavelets that are as smooth as we want, and that have as good bandpass characteristics as we choose. We can also construct wavelets with optimal time-frequency localization, starting from any given wavelet function.

SIGNIFICANCE: This work will provide the scientific community with new tools for the analysis and processing of data and images. The sampling theory has already been applied to the design of new tomographic reconstruction algorithms. It has also been applied to the enlargement and reduction of images. The multiresolution and wavelet representations are currently being applied at NIH to image coding, image transmission, and the rotational and translational alignment of autoradiograms.

PUBLICATIONS: Aldroubi A, Unser M. Multiresolutions and wavelets: algebraic structure and families in relation with Shannon's sampling theory and the Gabor transform. In: Fossum RM, ed. 1992 annual meeting of the American Mathematical Society. Baltimore: 1992 (in press).

Aldroubi A, Unser M. Families of wavelet transforms in connection with Shannon's sampling theory and the Gabor transform. In: Chui CK, ed. Wavelets--a tutorial in theory and applications. San Diego: Academic Press, 1992;509-28.

Aldroubi A, Unser M, Eden M. Cardinal spline filters: stability and convergence to the ideal sinc interpolator. Signal Processing 1992 (in press).

Aldroubi A, Unser M. Families of multiresolution and wavelet spaces with optimal properties. Numerical Functional Analysis and Optimization (in press).

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10404-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mathematical Methods in Gel Electrophoresis

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Akram Aldroubi, Ph.D.	Senior Staff Fellow	BEIP, NCRR
Michael Unser, Ph.D.	Visiting Fellow	BEIP, NCRR

COOPERATING UNITS *(if any)*
LTPB, NICHHD (M. Garner, A. Chrambach); DCRT

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Office of the Director

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Electrophoresis is a technique widely used in biology to separate, classify, and study complex mixtures of macromolecules and particles (such as DNA). We have applied mathematical methods and techniques to problems in electrophoresis. In particular, we have developed models describing the motion of macromolecules and virus-sized particles in gels. We have defined a resolution function which is well adapted to electrophoretic separation of particles such as DNA. The models of motion, together with the resolution function, allow us to predict the minimal time and optimal migration path for the resolution of complex mixtures, such as those encountered in DNA sequencing. We have also developed methods for evaluating the efficiency of various polymers in the separation of macromolecules of various sizes. For this purpose, we have defined the separation efficiency function, as well as the resolution efficiency function. These functions are used to evaluate different polymers relative to one another. These techniques give us a practical means by which to select the most efficiently sieving polymer solution for a particular molecular size. Finally, we have implemented the mathematical algorithms used in the analyses in a user-friendly software package for use on a personal computer.

OBJECTIVES: Our goal is to develop models, techniques, and methods for the quantitative analysis of gel electrophoresis. More specifically, we want to develop means by which to predict the way different particles separate in various media.

METHODS EMPLOYED: The motion of the particles is described by combining Ogsten's model of gel electrophoresis with the diffusion equation in partial differential equations. The resolution function is derived from a property of Gaussian functions. The time and migration path of particles in gels are deduced by combining the equations of motion with the resolution function. The efficiency function is deduced by using Ogston's model of particle mobility in gels for two particles with different radii, and letting the difference tend to zero.

MAJOR FINDINGS: Our models describe the motion of macromolecules and virus-sized particles in gels. The resolution function we have defined describes the degree of separation of a mixture of particle populations. The equations we derived predict the time length needed for separating a mixture under experimental conditions. The resolution efficiency function we have found allows us to compare the effectiveness of different media in sieving both large and small particles. Our theory has been applied to calculate the capillary electrophoresis of DNA sequencing separations. It showed that acceptable resolution could be obtained using shorter times than those commonly used in practice.

SIGNIFICANCE: A savings of time (by a factor of about 2.5) can be achieved in the separation of DNA mixtures. Thus, with our method, we can minimize the electrophoresis time in DNA sequencing, circumventing one of the limiting steps in electrophoresis. We have also applied our theory to evaluate four polymers relative to one another in terms of their efficiency in the separation of macromolecules of various sizes, and derived a nomogram that allows the practical selection of the sieving media and the conditions for the separation of molecules of a desired size range.

PUBLICATIONS:

Aldroubi A, Garner M. Minimal electrophoresis time for DNA sequencing. BioTechniques 1992 (in press).

Chrambach A, Aldroubi A. The relative efficiency of molecular sieving in solutions of four polymers. Electrophoresis (in press).

Aldroubi A, Unser M, Tietz D, Trus B, Chrambach A. Computerized methods for analyzing two-dimensional agarose gel electropherograms. Electrophoresis 1991;12:39-46.

Tietz D, Aldroubi A, Schneerson R, Unser M, Chrambach A. The distribution of particles characterized by size and free mobility within polydisperse populations of protein-polysaccharide conjuguates, determined from two-dimensional agarose electrophoretograms. Electrophoresis 1991;12:46-54.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10405-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
A System to Measure Head Motion Inside a PET Scanner

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Seth Goldstein, Sc.D. Chief, MES BEIP, NCRR

COOPERATING UNITS *(If any)*
Nuclear Medicine Department, Clinical Center (M. Green)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.6	0.6	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
_(a1) Minors
_(a2) Interviews

_(b) Human tissues

(c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

An optical system has been developed to measure the small motions of a person's head during a positron emission tomography (PET) scan in order to correct the scan and thereby improve the resolution of the image. The system uses a position-sensitive detector to sense changes sequentially in the positions of four blinking LEDs that are fixed to the patient's head. The computer-controlled instrument computes the six degrees of freedom (three angles and three positions) that are necessary to characterize head movement.

OBJECTIVES: To improve the resolution of PET scans, it is necessary to either eliminate motion of the head or correct for this motion. Since the first approach has been inadequate to date, the present system has been designed to measure head motion so that corrections can be made to the scan.

SIGNIFICANCE: The effective resolution of PET scans (~5mm) is degraded by approximately a factor of 2 due to the effects of head motion during a scan and between two scans taken an hour apart. Elimination of this additional uncertainty in measurements would represent a significant advance. The cost of the system under development is only a few per cent of the scanner's cost. Its use should put a minimal additional burden on the system operator, since it is under the control of a user-friendly computer.

METHODS EMPLOYED: Patient head motion is determined by optically measuring the positions of four blinking LEDs that are fixed to the head. A position-sensitive detector (PSD) is used. This device senses the x- and y-coordinates of the centroid of the light falling on its photosensitive surface. By using sequentially blinking, extremely bright LEDs under stable, relatively dark ambient conditions, the PSD output can be related unambiguously to the LED positions. The sensor electronics is under GPIB control, which in turn is controlled by a Macintosh computer running the program Labview. This program does the required computations to generate the three angles and three positions required to specify head motion. The computer also controls the instrument setup, thus relieving the technician of this burden.

PROPOSED COURSE: The hardware and software were completed and checked out recently, and the system will be installed shortly. The system will then be evaluated and modified as necessary. A patent is being pursued.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10406-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Development of a Fiber-Optic Gastric pH Sensor

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

John I. Peterson, Ph.D.	Chemist	BEIP, NCRR
Eden Netto, M.Sc.	Electrical Engineer	BEIP, NCRR

COOPERATING UNITS *(if any)*
None

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

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NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0.0

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects _(b) Human tissues (c) Neither

_(a1) Minors

_(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

A fiber-optic pH sensor and associated instrumentation are being developed for measurement and monitoring of intraesophageal and intragastric pH. Composed of replaceable sensors, an optical system, and electronic circuitry, the instrument should be able to measure pH in the range of 0-7, with an accuracy of 0.1 pH units and a response time in the range of 30 sec, for up to 24 hours. This instrument should be useful for the research, diagnosis, and evaluation of acid-related disorders of the upper gastrointestinal tract, and for studies on the influence of diet and antisecretory drugs.

OBJECTIVES: The objective of this work is to develop a dye-based sensor that covers a broad pH range from neutral to high gastric acidities. Several fiber-optic pH sensor systems have been developed, based on extensions of the approach originated here by Drs. Peterson and Goldstein, but with one exception they have not been for the low pH range. (The exception is a system intended for very high acidity measurements in nuclear fuel technology.)

METHODS EMPLOYED: The project consists of several areas of work:

1. A system of dyes must be developed and optimized which will have the correct optical and chemical characteristics (acidity constants) to cover the pH range of interest and provide suitable wavelength and absorbance properties.

2. This system must be combined with a dye support system having several intrinsic goals: to fix the dyes, so they will be stable; to provide a diffusion medium, so they will equilibrate with the surrounding pH; and to be formed into a probe system with a short response time. The possible toxicity of both the dye and the support system must be assessed.

3. A probe arrangement must be devised that will both be physically strong and have a container for the dye system that allows diffusion of hydrogen ions while limiting the flow of higher molecular weight substances. Possible container materials must be tested.

4. Model instrumentation must be developed that involves experimentation to combine an optical system with the probe and dye system. An instrument must be constructed for further testing of the performance of the pH probes. This step includes combining the optical system with electronic interfacing and computer programming.

5. When a working system is available, further *in vitro* testing and evaluation can be done for dye system optimization and testing of probe stability, accuracy, and precision. Testing of the probe in gastric material will be necessary to discover potential problems, such as interference from colored substances and possible destructive action on the probe.

6. If the *in vitro* testing does not show major problems, then *in vivo* testing in animals should proceed to insure that acceptable performance is achieved. This would be done in conjunction with electrode measurements for comparison. Some testing in humans would be desirable, if possible.

SIGNIFICANCE: This project represents a potential for significant contribution to biomedical engineering and clinical research, since current techniques involving electrodes are uncomfortable, cumbersome, and subject to difficulties due to electrode coatings and electrical safety hazards. A suitably

designed fiber-optic probe would be smaller, safer, and more reliable, and would facilitate diagnostic and research measurements with a patient-wearable system.

MAJOR FINDINGS: The first three areas of work listed above have been partially developed; test probes are being made and evaluated for their optical performance and response to test solutions of various acidities. Combinations of dyes and a polymeric dye-support system, along with a method of probe construction using ion-permeable tubing, are being tested.

PROPOSED COURSE: Work will continue for evaluation and optimization of the probe construction, as outlined in steps 4 and 5 above. Once a suitable system is available, a model instrument will be fabricated for further testing, optimization of the probe, and *in vivo* testing.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10407-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Elasticity and Active Force Generation in Cochlear Outer Hair Cells

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Richard Chadwick, Ph.D. Head, Theor. Biomech. Group BEIP, NCRR

COOPERATING UNITS *(if any)*
LCB, NIDCD (K. Iwasa)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
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INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, MD 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)
_(a) Human subjects _(b) Human tissues X (c) Neither
_(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

To establish the magnitude of the electromechanical force generated by the outer hair cell from the mammalian cochlea, we use a cylindrical membrane model characterized by area and shear moduli for a passive elastic element, and a membrane potential-dependent, active tension-generating element. We measured pressure-strain relations to determine the elastic moduli, and found the area modulus was both close to a lipid bilayer and an order of magnitude larger than the shear modulus. We also determined that the active tension element is nearly isotropic, with an amplitude sensitivity of about 2×10^{-2} $Nm^{-1}V^{-1}$. At 73 dB of acoustical stimulation, the active force generated per outer hair cell is about 0.6 nN--close to the force applied to a corresponding area of the basilar membrane by acoustic pressure. This finding supports the hypothesis that the outer hair cell acts as a feedback motor in the fine-tuning mechanism of the mammalian ear.

OBJECTIVES: The motility of the outer hair cell (OHC) from the mammalian cochlea is considered essential for fine tuning. Extensive efforts have been directed toward characterizing the motility mechanism. It has been established that it is membrane potential-dependent. A hyperpolarizing pulse elongates the cell, and a depolarizing pulse shortens it. Tight-sealed membrane patches, formed on the lateral wall, undergo area changes when the membrane potential is changed. A hyperpolarizing pulse increases membrane area, and a depolarizing pulse reduces it. Although these electrokinetic shape changes are well characterized, it has not been previously demonstrated that an OHC can generate sufficient force to influence the basilar membrane. We use a biomechanical membrane model of the OHC to investigate this issue.

METHODS EMPLOYED: Isolated outer hair cells from guinea pig cochlea, light microscopy, inflation by micropipette, mathematical modeling, and image analysis were used in this project.

SIGNIFICANCE: The magnitude of the active force is comparable to that due to acoustic loading. Hence, the OHC has sufficient strength to act as a feedback motor for fine tuning of the mammalian cochlea.

PROPOSED COURSE: Capacitance measurements are being carried out to further assess the magnitude of OHC surface area changes.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10408-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Perfusion System for Animals

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Elijah C. Walker, M.S. Section Chief ACES, BEIP, NCRR

COOPERATING UNITS *(if any)*
SB, DCT, NCI (A. Thom, D. Fraker, J. Norton)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

SECTION
Applied Clinical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.5	1.0	0.5

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects _(b) Human tissues X (c) Neither

_(a1) Minors

(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

New drug therapies are being developed for cancer treatment of isolated organs. Research on several animal models is being conducted prior to treatment of humans. Portable instrumentation is required for small animal surgery on the rat, and special catheters are required for liver isolation in the pig.

This project involves the design of a portable physiological monitoring/surgical system for liver perfusion in the rat, and design and fabrication of multilumen catheters for liver isolation in the pig.

OBJECTIVES: To design a portable physiological monitoring system with sufficient working space to perform surgery on rats. A special low-volume oxygenator is also required for isolated liver perfusion. The following parameters will be monitored: perfused blood oxygen saturation, body temperature, ECG, and blood pressure.

PROGRESS: Several systems have been designed and built: (1) Two special double-lumen catheter connectors of different sizes were built for the pig. Because of the surgical difficulty of using this technique, a different and less demanding approach was tried. (2) A small surgical/monitoring table was built for the rats. It included a pump (from a Pancretec™ microinfusion pump), pressure transducers, a blood reservoir and circuit, and a specially fabricated, hollow-fiber micropore tubing oxygenator with a Gould strip chart recorder, a thermistor for rectal temperatures, and a pulse oximeter for blood oxygen saturation, as well as a simulated appendage for perfused oxygen saturation. The instrumentation and oxygenator are performing very well.

PROPOSED COURSE: We are now replacing the strip chart recording system and the electronics with a MacIntosh computer and an instrumentation system from Biotrac, Inc., in California. Additionally, ECG (and possibly pH) will be monitored.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: Z01 RR 10409-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Quasi-Elastic Light Scattering in Multiply Scattering Media

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Joseph M. Schmitt, Ph.D. Staff Fellow BEIP, NCRR

COOPERATING UNITS *(if any)*
DCRT (R. Nossal)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

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INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

_(a) Human subjects
  _(a1) Minors
  (a2) Interviews

_(b) Human tissues

X (c) Neither

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

Several properties of dense optical materials can be probed using quasi-elastic light scattering (QELS) techniques. In this study, we have used QELS to probe photon path lengths in dense liquids containing particles that undergo Brownian motion. Potential applications include remote sensing of the size and concentration of biological macromolecules.

OBJECTIVES: To study basic aspects of light transport in multiply scattering media, using quasi-elastic light scattering to probe optical path lengths.

METHODS EMPLOYED: The time autocorrelation function, $I(\tau)$, of the backscattered intensity detected at the surface of a dense medium containing moving particles, contains information about the distribution of photon path lengths in the medium. We have developed a theory relating the photon path distribution to the shape of $I(\tau)$ measured in the reflectance mode. By applying cumulant analysis methods, the first and higher-order moments of the distribution can be derived from measurements of $I(\tau)$.

To validate the theory, we employed a simple experimental setup consisting of a He-Ne laser light source and a multichannel correlator. Suspensions containing different concentrations and sizes of polystyrene latex beads were prepared to model biological specimens having a range of optical scattering and absorption coefficients. The values of the mean photon paths, obtained from cumulant analysis of the measured autocorrelation functions, were compared with those predicted by theory for the known sizes and concentrations of the beads.

In a separate study, we used the newly developed theory and methods for testing scaling relationships to describe photon migration in anisotropically scattering media. This was easily accomplished by modifying the experimental setup and procedures slightly.

MAJOR FINDINGS: Excellent agreement was found between the predicted and experimental values of the first moment of the autocorrelation function over a wide range of optical densities. These results provide further evidence of the validity of the concepts underlying the heuristic random-walk and photon-diffusion models of light transport in optically dense media.

However, we found a discrepancy between the predicted and observed scaling of the mean photon path lengths, measured as a function of the anisotropy parameter of the scattering particles in a dense medium. This discrepancy prompted us to reexamine the theoretical assumptions.

SIGNIFICANCE: Although statistical parameters of photon migration in optically dense media can be measured by time-of-flight spectrophotometry, few laboratories can afford the equipment needed to apply this technique. We have demonstrated in this study that QELS techniques provide relatively inexpensive alternatives to time-of-flight spectrophotometry in some applications. The QELS techniques developed in this study have enabled us to answer fundamental questions regarding the transport of light in optically dense tissues.

PROPOSED COURSE: In the future, we will attempt to characterize living samples by using QELS techniques. A biological tissue of particular interest is cartilage, which consists of mobile macromolecules trapped in a rigid matrix.

PUBLICATIONS: Nossal R, Schmitt JM. Measuring photon path lengths by quasi-elastic light scattering in a multiply scattering medium. Proc SPIE 1991;1430:37-47.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE

NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER: ZO1 RR 10410-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
MRI Muscle Dynamometer

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*

Stephen B. Leighton, Sc.D.	Mechanical Engineer	MES, BEIP, NCRR
Thomas R. Clem, Sr., BSEE	Electrical Engineer	EEES, BEIP, NCRR

COOPERATING UNITS *(if any)*
IR CE, NHLBI (T. Ryschon)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

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Mechanical Engineering Section

INSTITUTE AND LOCATION
NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
0.5	0.4	0.1

CHECK APPROPRIATE BOX(ES)

X _(a) Human subjects
_(b) Human tissues
(c) Neither
_(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

It is useful to be able to make *in vivo* metabolic measurements (e.g., of muscle phosphocreatinine) with magnetic resonance spectroscopy while subjects are exercising specific muscle groups on a dynamometer. It is the purpose of the present work to design and construct a programmable wrist- and ankle-joint dynamometer for use in the magnetic environment of the NIH 4-Tesla MRI instrument.

OBJECTIVES: Human studies of muscle energy metabolism during exercise have traditionally relied on chemical analysis of muscle samples obtained immediately following a trial of muscular work. Such tissue samples are acquired through a percutaneous approach to an easily accessible muscle mass, and must be limited in number to avoid functional disruption of the muscle. In addition, ethical considerations have precluded the study of muscle from children using this technique. ^{31}P-NMR spectroscopy offers a noninvasive alternative to muscle biopsy for the measurement of high-energy phosphate concentration (ATP, Pi. ADP, PCr). In addition to being noninvasive, measurements can be acquired during exercise, and several parameters can be measured simultaneously, giving greater insight into the energetic processes involved in muscle contraction. Meaningful studies of muscular work require careful control and measurement of muscle tension. Bicycle ergometers, treadmills, and kinetic dynamometers are commonly used in the exercise physiology laboratory, but cannot be used in the NMR experiment for several reasons. The high-strength (4-Tesla) magnetic field required for NMR measurements precludes the use of any ferrometallic devices within 10 to 12 feet of the magnet. NMR measurements require the muscle to be placed in the center of the magnetic field, which is found midway inside the cylindrical bore of the magnet (5 meters in length and 70 cm in diameter). Thus, the dimensions and composition of any ergometer for use in NMR studies of humans must be unique, and cannot be satisfied by commercially available devices. An ergometer is being designed (and will be constructed) by the BEIP to meet these critical specifications. The ergometer will be constructed to allow use with children, since this group of humans has not been adequately studied. The first implementation of the ergometer will be to study the high-energy phosphate concentration of contracting forearm muscle in children at different stages of maturity and at different levels of physical conditioning. For this study, a forearm module will be coupled to the main ergometer, allowing precise control of the resistance to forearm flexion and extension, and precise measurement of the force produced in opposing this resistance. In subsequent studies, a leg module will be implemented to study the energetics of flexion and extension of the foot. The ergometer will provide state-of-the-art, unprecedented control of the exercise being investigated.

We intend to create a system that is able to subject the ankle or wrist joints to programmed torques, position functions, or velocities. Magnitudes of the parameters will be adjustable to either adult or pediatric subjects. Torques will either resist or abet muscle motion, allowing both eccentric and concentric work to be studied. All parts of the system within the MRI magnet will be nonmagnetic, in order to avoid any interference with the spectroscopic measurements. All system controls and all logging of data will be handled by a remote personal computer.

METHODS EMPLOYED: A DC servo motor system will be mounted at a sufficient distance from the magnet so that the MRI field and the

iron of the motor will not interact significantly. Velocities and torques will be transmitted to the interior of the magnet with a high-stiffness, low-inertia shaft system. Torque will be monitored by a custom fiber-optic torque sensor. A dedicated servo-controller will receive position and torque feedback and overall commands from the personal computer.

MAJOR FINDINGS: The device is in the design stage at this time.

SIGNIFICANCE: This apparatus will permit new studies of *in vivo* human muscle metabolism during specific exercise regimens, for elucidating new information on muscle mechanics and biochemistry.

PROPOSED COURSE: The design will be finished and the apparatus constructed.

DEPARTMENT OF HEALTH AND HUMAN SERVICES - PUBLIC HEALTH SERVICE
NOTICE OF INTRAMURAL RESEARCH PROJECT

PROJECT NUMBER
Z01 RR 10411-01 BEI

PERIOD COVERED
October 1, 1991 to September 30, 1992

TITLE OF PROJECT *(80 characters or less. Title must fit on one line between the borders.)*
Mapping Electrophysiological Signal Sources into 3-D Brain Images

PRINCIPAL INVESTIGATOR *(List other professional personnel below the Principal Investigator.) (Name, title, laboratory, and institute affiliation)*
Binseng Wang, Sc.D. Visiting Scientist BEIP, NCRR

COOPERATING UNITS *(if any)*
HMCS, DMNB, NINDS (C. Toro, T. Zeffiro, E. Wassermann, R. Thatcher); CNB, NINDS (M. Hallett)

LAB/BRANCH
Biomedical Engineering and Instrumentation Program

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NCRR, National Institutes of Health, Bethesda, Maryland 20892

TOTAL STAFF YEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	0.0

CHECK APPROPRIATE BOX(ES)
X _(a) Human subjects _(b) Human tissues (c) Neither
_(a1) Minors
(a2) Interviews

SUMMARY OF WORK *(Use standard unreduced type. Do not exceed the space provided.)*

While there are several imaging technologies that offer spatial resolution in studying the brain, none can provide the temporal resolution of electrophysiological techniques. Conversely, the latter cannot compete with the former in terms of anatomical detail. Therefore, there is a natural desire to integrate different modalities of data and images for the study of brain function and the diagnosis of disorders. The Human Motor Control Section of the NINDS has been using EEG, MEG, transcranial magnetic stimulation (TMS), MRI, and PET to study voluntary movement for several years. A method for integrating several or all of these approaches was considered highly desirable. A method was developed for mapping equivalent dipole sources, computed from EEGs and MEGs, into MRI and PET. Later, this method was extended to include scalp maps obtained by TMS of the brain. The precision of the mapping method that we developed is 3 mm, which is adequate for integration of the various signals.

OBJECTIVES: To develop a method for mapping dipole sources that can be computed from electrophysiological signals, such as EEG and MEG, into 3-D brain images, such as MRI and PET. The method has been extended to include TMS scalp maps.

METHODS EMPLOYED: As dipole sources are usually calculated using spherical head models, a means was needed to account for variations in EEG electrode positions and individual head size and shape. A magnetic digitizer was used to acquire both the 3-D coordinates of the electrode positions and about 300 points from each subject's head. These electrode positions, and the center and radius of the sphere that best fit the scalp points, were introduced into the dipole models. The sources thus computed were transferred from the sphere into the digitized head, using scalp landmarks as references. To map the sources from the digitized head into the MRI, we used a surface registration algorithm to align the digitized head surface with the MRI head contours of the same subject. The transformation parameters obtained from the registration were then used to map the sources into MRI. Similarly, PET images were integrated by registering their brain contours with the corresponding contours obtained from the MEIs. Finally, the MRIs (with the sources mapped into them) were overlaid on the PET images to provide a consilidated image of electrophysiological data with anatomical and physiological images.

MAJOR FINDINGS: Sources computed from voluntary finger movement-evoked EEGs and MEGs, and mapped into MRI and PET, show that it is possible to localize dipoles within 17 mm, on average, of the PET peaks for the contralateral motor cortex. Less accuracy (about 35 mm) was found for secondary dipoles. TMS projections were found to pass within 13 mm, on average, of the PET peaks. Both results are believed to be the first ever obtained.

SIGNIFICANCE: The comparison of different modalities of data and images will allow researchers to evaluate and compare each modality more precisely, and to formulate new hypotheses for testing. For example, the adequacy of different models available for dipole sources computation can be evaluated by comparing the source locations and orientations with MRI and PET. Similarly, one can judge distinct techniques used to obtain TMS scalp maps of muscle excitation. Conversely, PET can provide *a priori* information about where dipole sources should be located, and thus help to extract more information from source models. More interesting, however, is the possibility of using the images to decide where and how one can stimulate and record from specific areas of the brain. In the future, it might be possible to use multimodality integration to differentiate precisely between healthy and abnormal tissue in the brain. This would ease the tasks of neurosurgeons significantly.

PUBLICATIONS: Wang B, Toro C, Zeffiro TA, Nagamine T, Hallett M. A method for mapping electrophysiological sources into brain tissues. In: O'Connell SM, ed. Program of Science Innovation '92. San Francisco: American Association for the Advancement of Science and Science Magazine, 1992;89.